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Content of Carrageenan, Chlorophyll *a* and Carotenoid of *Kappaphycus alvarezii* Cultivated in Different Seawater Depth Laikang Village, District of Mangarabombang, Takalar Regency

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Abstract

The objective of the study is to analyze the content of carrageenan, chlorophyll *a* and carotenoid of seaweed *K. alvarezii* cultivated in different seawater depth. The research consists of 5 treatments of different sea level, which are 20 cm; 100 cm; 200 cm; 300 cm and 400 cm, each treatment consists of three re trials, then there will be 15 trial test. The method used is by randomly hanging seaweed in vertical order. Variance analysis is performed at the rate of 95%. The findings show significant difference of depths for cultivation towards carrageenan content and insignificant difference towards chlorophyll *a* and carotene content of seaweed. The highest carrageenan content is in the depth of 20 cm (56,31%) and the lowest is in the 400 cm (17,10%). Chlorophyll *a* and carotenoid content is relatively high in the depth of 100 cm (respectively 0,013 mg/g^{-1} and 0,299 mg/g^{-1}) and the lowest is in the depth of 20 cm (respectively 0,006 mg/g^{-1} dan 0,163 mg/g^{-1}).

Keyword: Content of carrageenan, chlorophyll *a*, carotenoid, Depth of cultivation, Seaweed

1. Introduction

Kappaphycus alvarezii has been widely cultivated in various locations. However, limitation of farming area in the shallow seawater is the main cause for conflict between farming area usage as for not all locations have appropriate depth for culture. Besides, the use of deep seawater will certainly cause problems, as for it has more complex physical chemistry characteristics compared to shallow seawater, so it will need much more energy to change it to become useful.

Aspect expected to overcome quality and quantity problems of seaweed is ecological factors in the cultivation of *K. alvarezii*, mainly related to water depth for the culture. Results show that environmental factors play important role mainly temperature, salinity, nutrient, and light, and other ecological factors (Gerung & Ohno, 1997; Aks & Azansa, 2002). Besides, depth of cultivation is another factor taken apart (Nurdiana et al., 2008; Naguit et al., 2009; Naguit & Tisera, 2009). Currently, application of *K. alvarezii* culture by making use seawater depth is one of the alternatives for cultural media expansion and also a way to make use of deep seawater (Sahoo & Ohno, 2003).

Difference in sea level depth causes variation in light intensity of each zone so that caused difference in the growth of tallus as a measurement for seaweed growth. Increase of photosynthetic process will cause metabolism proses then stimulate seaweed to absorb more nutrients, this will help seaweed to grow better. Besides, difference of light intensity and nutrients will lead to morphological difference, difference of chlorophyll *a* and carotenoid content. This cause to the increase of seawater depth and incoming light intensity will decrease which then lead to further decrease of the speed in photosynthetic process of the plant. Photosynthesis produce polysaccharide compound which is part of carrageenan. If photosynthetic process experience disturbance then it will cause abnormal growth of seaweed and carrageenan content.

Based on the above explanation, it will need to conduct a research observing seaweed cultivation of different seawater depth towards content of carrageenan, chlorophyll *a* and carotenoid in *K. alvarezii*. The objectives of the study is to analyze the content of carrageenan, chlorophyll *a* and carotenoid of *K. alvarezii* cultured in different seawater depths. The research is expected to be able to provide information on optimum depth of seaweed culture in order to increase quality of the content of carrageenan, chlorophyll *a* and carotenoid.

2. Research Method

2.1 Location and Time of Research.

This research was performed in center for seaweed culture Puntondo, Laikang village (LS 05˚35.828' dan LU 119˚29.367'), District of Mangarabombang, Takalar Regency. Analysis of the content of chlorophyll *a* and carotenoid was performed in Water Quality Laboratory University of Hasanuddin Macassar, South Sulawesi. This was conducted for 3 months, starting from April to June 2011.

2.2 Materials of Research

Object used in the research was seaweed seeds of *K. alvarezii* taken from the surrounding area of the research. Materials used are; PE plastic rode (Ø No. 8, 4, 2 mm), anchor of sand filled sack, sliding bar, scale and float ball. This experiment consisted of 15 trial units, each of them with initial weight of 50 g per seaweed knot in the plastic rod of PE No. 2 mm.

2.3 Experimental Design and Data Analysis

This study is based on the complete random design with 4 treatments and 3 replicates (Gaspesz, 1991). Tied seeds is hang in random vertical way on plastic rode of PE No. 5 mm with different intervals of cultivation , they are; (A) cultural depth of 20 cm, (B) cultural depth of 100 cm, (C) cultural depth of 200 cm, (D) cultural depth of 300 cm, and (E) cultural depth of 400 cm of sea surface. Plant was harvested when seaweed is of 49 days after the plantation. Measurement of carrageenan content from dried sample was performed by referring to procedures used in the analysis of carrageenan content is NaOH extraction method (Yasita & Rachmawati, 2008) using the following formula:

$$Carrageenan\ Content\ (\%) = \frac{Weight\ of\ Carrageenan\ Fiber}{Weight\ of\ Dried\ Sample} \times 100 \quad (1)$$

Analysis of chlorophyll *a* and carotenoid content is as below:

1. 500 mg of tissue was crushed in 10 ml acetone 80% till homogeneous.
2. Centrifugated in the speed of 3000 rpm for 15 minutes to obtain supernatant.
3. Particles of supernatant re extracted by washing it using 5 ml acetone 80% till colorless.
4. Extract was used to determine photosynthesis pigment based on the absorption capacity 645 nm and 663 nm, and carotenoid extract is measured at absorption capacity of 480 nm from a spectrophotometer.

Analysis of chlorophyll *a* content was performed based on a method by Arnon (1949; in Thirumaran & Anantharaman, 2009). Extract of chlorophyll *a* is calculated using below formula:

$$\text{Chlorophyll a (mg/g)} = \frac{12.7 \times A663 - 2.69 \times A645}{a \times 1000 \times W} \quad (2)$$

Description:

A = Absorbent of each wave length

V = Volume of solvent material (ml)

W = Weight of seaweed talus (g)

Meanwhile, to determine number of carotenoid content estimated in talus, it is used a method

by Kirk (1965; in Thirumaran & Anantharaman, 2009). Extract of carotenoid content is calculated using below formula:

$$Carotenoid\ (mg/g) = \Delta A\ 480 + (0{,}114 \times \Delta A\ 663) - (0{,}638 \times \Delta A645) \quad (3)$$

Design used in this research is Complete Random Design (CRD). Influence of various cultural depth of *K. alvarezii* is examined towards chlorophyll *a* and carotenoid content. To know the influence of cultural depth towards carrageenan, chlorophyll *a* and carotenoid content in *K. alvarezii*, then performed a test of ANOVA in insignificant rate of 95% (Steel & Torrie, 1980). If influence is found, then followed by further test Tukey and used SPSS version 16.00 for analysis.

3. Result and Discussion

3.1 Content of Carrageenan

Carrageenan content in *K. alvarezii* of different cultural depth in the end of the research is presented in Table 1.

Table 1. Mean of Carrageenan Content (%) of *K. alvarezii* in Difference Cultural Depth

Depth of culture (cm)	Content of carrageenan (%)±SD
20	56.31±5.30
100	38.70±2.74
200	27.29±3.41
300	33.74±1.64
400	17.10±3.94

Result shown in Table 1 figures that different seawater depth for cultivation show significant difference ($P < 0.05$) to the carrageenan content of *K. alvarezii*. Carrageenan content in depth of 20 cm (56.31%) is higher if compared to the depth of 100 cm (38.70%); 200 cm (27.29%); 300 cm (33.74%), and depth of 400 cm (17.10%). Carrageenan of 20 cm cultural depth showed the highest carrageenan content, while the cultural depth of 100 cm, 200 cm, 300 cm and 400 cm showed low content of carrageenan compared to the one of 20 cm. The high content of carrageenan in the depth of 20 cm is caused by optimum light intensity come through the sea level which is sufficient for formation of carrageenan through photosynthetic process.

Relationship between seawater depth and carrageenan content is of linear relation with regressive equation $Y = 52.09\text{-}0.085x$ ($R^2 = 0.76$), as presented in Figure 1. It shows strong correlation, in which 76 % of cultural depth shows strong influence on carrageenan content.

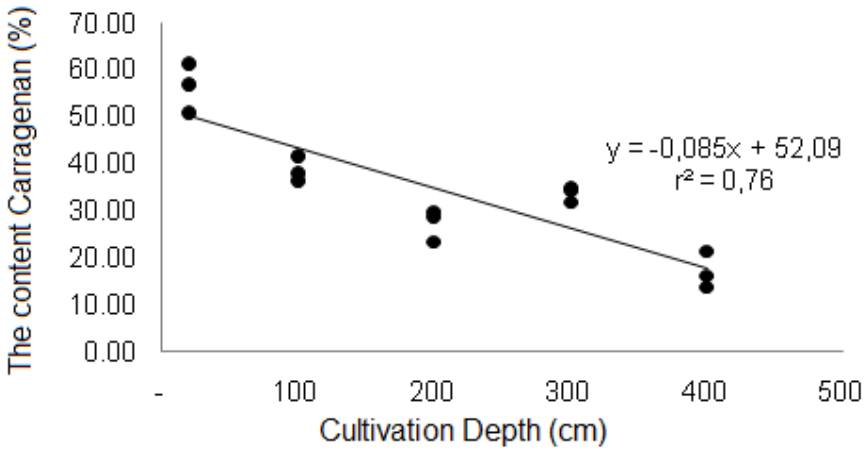

Figure 1. Graphic of relation between cultural depth and carrageenan content

3.2 Content of Chlorophyll a and Carotenoid

Means of chlorophyll *a* and carotenoid content of *K. alvarezii* cultivated in different seawater depths respectively presented in Figure 2 and 3.

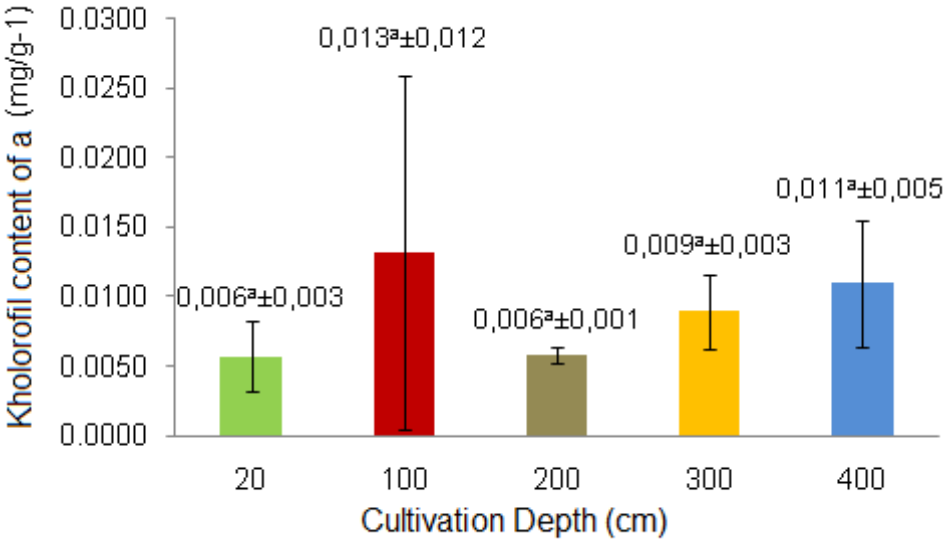

Figure 2. Histogram for mean of chlorophyll content of seaweed in each cultural depth

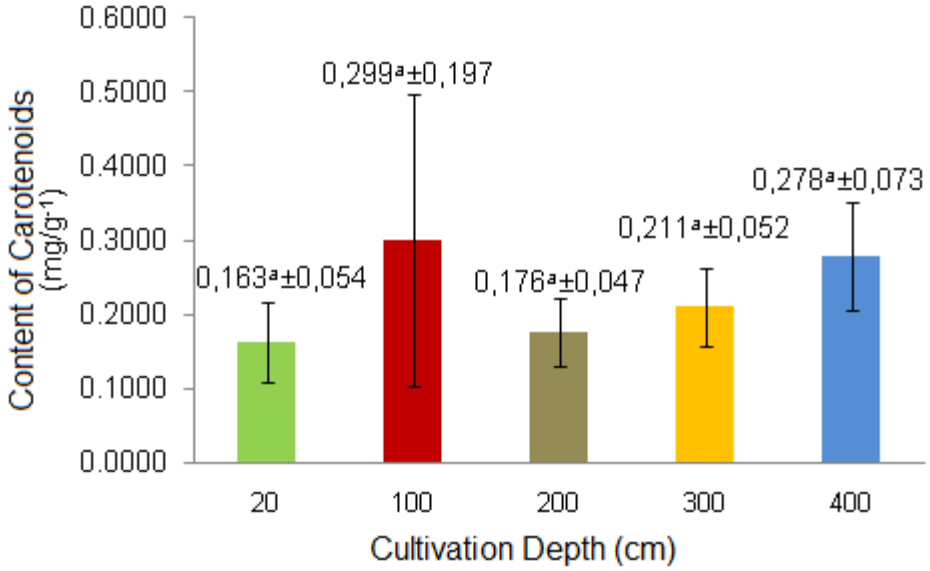

Figure 3. Histogram for mean of carotenoid content of seaweed in each cultural depth

Result of variance analysis shows that cultural depth reveal insignificant difference ($P > 0.05$) towards chlorophyll *a* and carotenoid content of *K. alvarezii*. Based on the findings, it show that cultural depth does not influence chlorophyll *a* and carotenoid content of yielded (Figure 2 and 3). This is in line with the opinion of Naguit and Tisera (2009) who determined the content of chlorophyll a in seaweed which showed insignificant difference with the depth. However, chlorophyll *a* and carotenoid content in *K. alvarezii* is relatively higher produced in cultural depth of 100 cm (respectively 0.013 mg/g^{-1} and 0.299 mg/g^{-1}), due to the high Mg (Magnesium) content in the seawater of 100 cm depth. Mg is an essential component in a synthesis for chlorophyll formation (Mainon, 2000 in Naguit & Tisera, 2009). Carotenoid content of *K. alvarezii* is in line with chlorophyll *a* content. This is because of the carotenoid content is an accessory pigment which protect chlorophyll *a* from damage. It occurred damage of chlorophyll *a* and carotenoid at 20 cm of sea surface caused by excessive light intensity and ultraviolet radiation know as photoinhibition and photodamage and also photooxidation due to extreme light intensity (Gleen & Doty, 1981). Meanwhile, based on the opinion of Peni et al. (2003), it mantioned the existence of disturbing inhibitor in photosynthesis and respiratory system, for example high light intensity would decrease nitrate reductase activity. The content of photosynthetic pigment is greater with the increasing depth (Ramus et al., 1976). Seaweed live in deep seawater level will receive few light with effective wavelength absorbed by chlorophyll (violet and red) that stimulate process of photosynthesis.

Based on Gantt and Cunningham (2001), chlorophyll could not accumulate when carotenoid synthesis is in the process, beta carotene is inhibited. Main function of yellow and orange pigments in photosynthesis is to protect photooxsidant of the excessive light intensity absorbed by surface light intensity.

Relationship between cultural depth and chlorophyll *a* content shows a weak correlation with regressive equation $Y = 0.004+1E\text{-}05\ X$ ($R^2 = 0.40$), as presented in Figure 4.

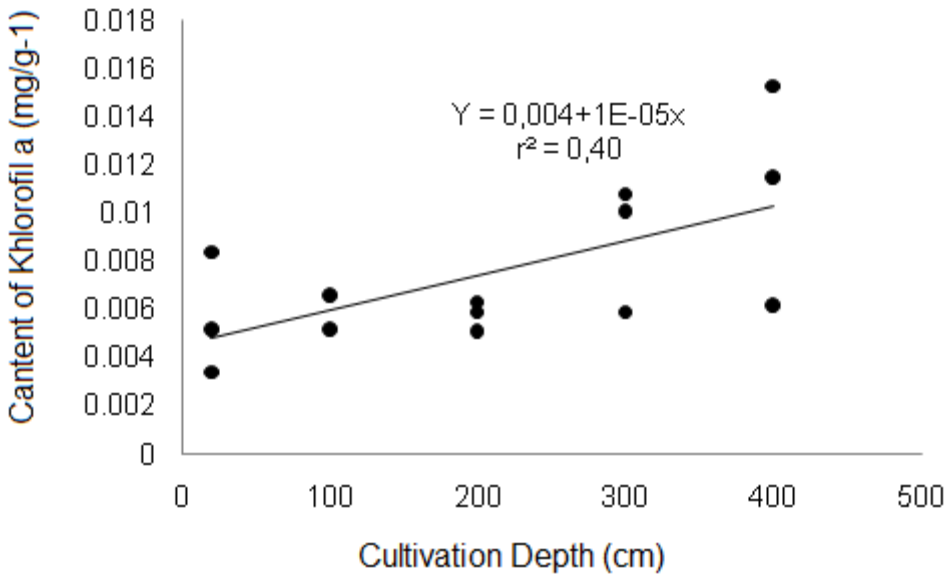

Figure 4. The relation shows a weak correlation in which 40% of cultural depth influence chlorophyll content

Relationship between cultural depth and carotenoid content is of weak correlation with regressive equation Y = 0.146+0.000 X (R^2 = 0.37), as presented in Figure 5. The relation pictures a weak correlation in which 37% of cultural depth show influence for carotenoid.

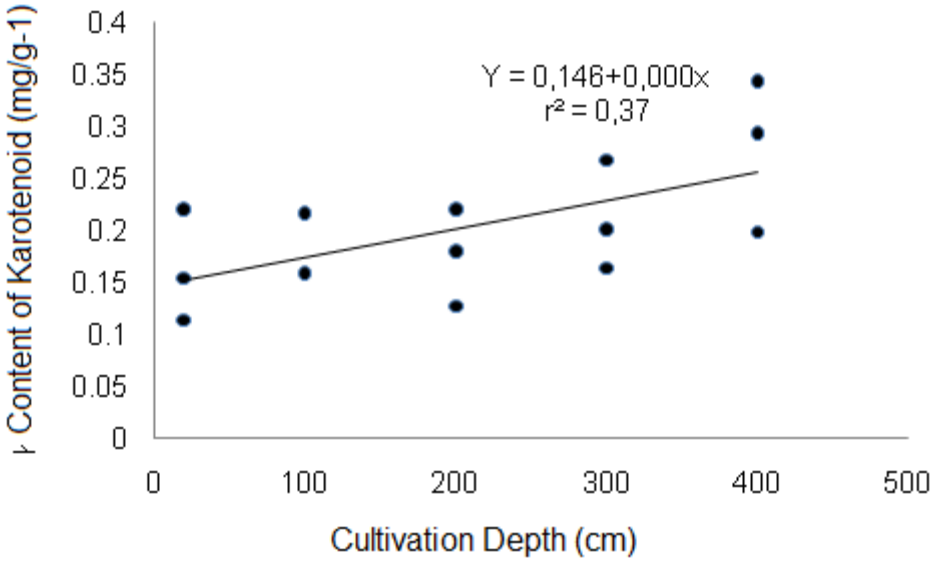

Figure 5. Relation of cultural depth and carotenoid content

4. Conclusion and Advice

a) The depth for *K. alvarezii* cultivation is significantly different to the content of chlorophyll *a* and carotenoid.

b) The lower carrageenan content in the cultural depth of 100 cm compared to 20 cm is caused by the high (not optimum) content of nitrogen in the cultural depth of 100 cm compared to the other cultural depths.

c) Content of chlorophyll *a* and carotenoid is higher in the depth of 100 cm compared to the depth of 20 cm.

d) Optimum light intensity for synthesis of chlorophyll and magnesium happens in cultural depth of 100 cm, while cultural depth of 20 cm chlorophyll is damaged by photodamage, photooxidation and photoinhibition (inhibition of chlorophyll synthesis by excessive light intensity).

e) Culture of *K. alvarezii* is suggested in the depth of 20 cm to obtain highest carrageenan content.

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