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The Effect of maceration period on contents and color brightness of phycoerythrin from *Gracilaria* sp.

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Abstract. Natural pigment from seaweed, is currently required by humans as one of active compounds that are usefull in the field of health, cosmetics and food. One of the pigments which potentially can be developed is red phycobilin pigment. The pigment can be obtained from *Gracilaria* sp. through maceration method. This research aims to find out wheter there is influence from the long maceration time toward to the content and the phycoerytrin color brightness of *Gracilaria* sp. The reseach method used experimental experiment with complete randomized design (CRD) which was consisted of nine treatments of long time maceration period three replications. Data analysis was used the Analysis of Variance (ANOVA) and continued to Duncan's Multiple Distance Test to. The result showed that treatments with the time of maceration for 30 hours was the best treatment on phycoerytrin content about 0.98 mg/g with the brightness value was 19.73 L*, reddish value was 7.23 a*, and yellowish value was 2.87 b* of *Gracilaria* sp.

1. Introduction

Pigment in seaweed consists of the main pigments which are chlorophyll and two additional pigments (accessories) in the form of carotenoids and phycobylin. The Main pigment has function for photosynthesis while additional pigments are used to absorb sunlight during photosynthesis process [1]. Other than used by seaweed for photosynthesis, the pigment is currently used by human as one of the active compounds useful in the field of health, cosmetics, and food [2]. One of the pigments that are potential to be developed is phycoerythrin, which is a group of phycobylin protein pigment with red color [3]. Phycoerythrin has potential as natural red food coloring, cosmetics, and also as a source of antioxidants in health supplements [4]. This pigment can be obtained from the macroalgae Rhodophyta, and one of them is *Gracilaria* sp. [5].

Phycoerytrin can be obtained from *Gracilaria* sp. through extraction with maceration method. This method is selected because phycoerythrin has characteristics that are easily degraded by high intensity of direct light and heat [6]. Maceration is extraction method with sample immersion in suitable solvent where active compounds can be obtained without heating process, so it will guarantee the active compound that



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are extracted will not be damaged [7]. Maceration of *Gracilaria* sp. needs an effective maceration time to separate the pigment algae cell so it will affect to the obtained phycoerythrin [8]. This is because the longer time of solvent agent contact with sample than the more phycoerythrin will be dissolved [9]. Research on extraction of phycoerythrin from *Gracilaria* sp. with 24 h maceration has been done and it could obtain phycoerythrin as much as 0.24 mg from every gram of sample [10]. Therefore, in this research, maceration method was used but with longer immersion time to know the maceration time that yield the highest phycoerythrin content and the lowest color brightness from *Gracilaria* sp.

2. Methodology

2.1. Time and place of research

This research was held at Educational Laboratory of Faculty of Fisheries and Marines, Airlangga University, Surabaya included maceration of *Gracilaria* sp., determination of phycoerythrin contents, and observation of phycoerythrin color brightness.

2.2. Tools and ingredients

Tools used in this research were dark colored bottle, scissors, mortar and crusher, digital scales, measuring cup, pH meter, refrigerator, UV-Vis spectrophotometer, centrifuge, and color reader. Materials used in this research were red algae *Gracilaria* sp., distilled water, acetone, and phosphate buffer solution with pH 6.8 ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and KOH).

2.3. Working procedures

Seaweed was obtained from traditional farmers at Jabon Village, Sidoarjo. Obtained seaweed was then inserted into polybags and stored in cool box filled with ice. Seaweed was then washed from dirt, cut into small pieces and then crushed with mortar and crusher. To make solution of buffer phosphate with pH 6.8, it was started by dissolving 13.79 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in distilled water till the border line of 1 L measure cup. Then, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ solution was added by KOH gradually till pH 6.8 [11]. Two grams of *Gracilaria* sp. wet sample that was already crushed with mortar and crusher were inserted into the test tube. After that, 5 mL of phosphate buffer solution was given to the extracts the phycoerythrin, while for chlorophyll extraction 5 mL of acetone were given [10]. Next, samples were being macerated according to the treatments (6, 12, 18, 24, 30, 36, 42, and 48 h) and stored in the refrigerator at 4 °C [12]. Sample without maceration (0 h) was directly calculated for the content of phycoerythrin and chlorophyll.

2.4. Test procedures

a. Calculation of *Gracilaria* sp. Phycoerythrin Content

Phycoerythrin content was calculated from every solvent used, based on the following formula:

$$\text{mg/ml Phycoerythrin} = 0.1247[(A_{564} - A_{730}) - 0.4583(A_{618} - A_{730})] \quad (1)$$

Description:

A = Absorbance value at the set wavelength

b. Calculation of *Gracilaria* sp. Chlorophyll Content

Chlorophyll content was calculated following the formula from [13] to know the concentration of the chlorophyll in every solvent:

$$\text{mg/ml Chlorophyll } \alpha = (11.75 \times A662) - (2.350 \times A645) \quad (2)$$

Description:

A = Absorbance value at the set wavelength

c. Calculation of Phycoerytrin Production and Chlorophyll of *Gracilaria* sp.

Calculation of Phycoerytrin Production and Chlorophyll were obtained to know the phycoerytrin and chlorophyll content in every sample:

$$\text{Pigment Production } \left(\frac{\text{mg}}{\text{g}} \right) = \frac{C \times V}{M} \quad (3)$$

Description:

C = Phycoerytrin Content (PE) or Chlorophyll (Chl) (mg/mL)

V = Extract volume of phycoerytrin or chlorophyll (mL)

M = Sample weight (mg)

2.5. Data Analysis

This research used Completely Randomized Design (CRD) consisted of nine treatments and three replications. Treatment in this research was maceration time for 0, 6, 12, 18, 24, 30, 36, 42, and 48 h to obtain phycoerytrin from *Gracilaria* sp. Data analysis in this research used Analysis of Variance and continued with Duncan's Multiple Range Test to determine which treatments gave different effects [14].

3. Results and Discussion

Table 1 shows that every treatment of maceration time gave significantly different effect to phycoerytrin content ($p < 0.05$). Statistical analysis was then continued to Duncan test and it showed that the highest phycoerytrin content on *Gracilaria* sp. was obtained from treatment I (48 h maceration) with 1.56 mg/g, while the lowest phycoerytrin content was obtained from treatment A (0 h) with 0.41 mg/g. According to [7], longer time of maceration will increase the extraction volume. This is because longer maceration time allows longer contact of solvent with sample, so it will balance concentration of the solution inside and outside of the extracted ingredients [15]. Table 2 shows that every treatment of maceration time gave significantly different effect to phycoerytrin content ($p < 0.05$). Analysis was then continued with Duncan test and the results showed that the maceration time that gave bright color phycoerytrin was treatment F (30 h) with high value of brightness and redness. High value of redness indicated high phycoerytrin got dissolved during maceration. Immersion for 30 enough provided sufficient time to dissolve phycoerytrin maximally. Limit time of contact happened after the creation of equilibrium states in the cell to dissolve phycoerytrin which is increasing the maceration time to dissolve other pigment. Improved maceration time dissolved other pigments so it affected the increase of dissolved pigment content and the decrease of obtained color brightness.

Table 1. Phycoerytrin content of *Gracilaria* sp. (mg/g) at different maceration time.

Treatment (hour)	Average of phycoerytrin content (mg/g)
A (0)	0.41 ^a ± 0.02
B (6)	0.58 ^{ab} ± 0.03
C (12)	0.62 ^{ab} ± 0.02
D (18)	0.74 ^{bc} ± 0.28
E (24)	0.95 ^{cd} ± 0.05
F (30)	0.98 ^{cd} ± 0.26
G (36)	0.99 ^{cd} ± 0.16
H (42)	1.04 ^d ± 0.08
I (48)	1.56 ^e ± 0.01

Note: different alphabet notation on the graphic shows significant differences among treatments (p<0.05).

There are other factor that can affect the increase of phycoerytrin content during maceration other than contact time and types of solvent; they are light intensity, solvent, pH and temperature during maceration. chromoprotein (polypeptide α and β) as pigment composer is very sensitive to temperature, light intensity, and pH so damaged done on the cell is not followed by pigment denaturation during extraction [16]. Those factors can affect the content of phycoerytrin and color brightness.

Table 2 Phycoerytrin Color Brightness of *Gracilaria* sp. (L*) in different maceration time.

Treatments (hour)	Average value of brightness (L*)	Average value of reddish (a*)	Average value of yellowish (b*)
A (0)	22.57 ⁱ ± 0.06	5.10 ^b ± 0.17	2.43 ^a ± 0.06
B (6)	19.20 ^d ± 0.00	6.00 ^{de} ± 0.10	2.77 ^b ± 0.15
C (12)	19.03 ^c ± 0.06	5.87 ^{cd} ± 0.15	2.77 ^b ± 0.11
D (18)	19.97 ^g ± 0.15	5.67 ^{cd} ± 0.25	3.33 ^{cd} ± 0.15
E (24)	18.57 ^b ± 0.06	5.50 ^c ± 0.10	3.30 ^{cd} ± 0.10
F (30)	19.73 ^f ± 0.06	7.23 ^f ± 0.06	2.87 ^b ± 0.06
G (36)	20.47 ^h ± 0.12	5.97 ^{de} ± 0.11	4.23 ^c ± 0.15
H (42)	19.43 ^e ± 0.06	6.47 ^e ± 0.06	3.53 ^d ± 0.06
I (48)	15.53 ^a ± 0.15	2.97 ^a ± 0.25	3.17 ^{bc} ± 0.23

Note: different alphabet notation on the graphic shows significant differences among treatments (p<0.05).

Treatment I (48 h) had the highest phycoerytrin content and the lowest brightness value (L*) , but it was not followed by increase in high reddish value (a*). Obtained a* value was low and close to greenish scale value (0 to -80), so it could be stated that the dissolved pigments was not only phycoerytrin. Dissolved pigments could be chlorophyll, carotenoids, as well as other phycobylin. Other dissolved pigments other than phycoerytrin can affect the decrease of brightness value because there were other color additions. *Gracilaria* sp. has red colored thallus that is the main characteristic of phycoerytrin pigment, but *Gracilaria* sp. also has chlorophyll pigment as its main pigment [4]. Chlorophyll is the main pigment found in every organism that performs photosynthesis [17]. Rhodophyta, a phycoerytrin pigment, will absorb sunlight and forward it to chlorophyll α to maximize photosynthesis process [1]. This is because the ability to chromatic adaptation of Rhodophyta to change characteristic of light absorption

during photosynthesis according to light source in the environment. After that, the pigment will absorb the strongest wavelength to become the dominant pigment.

Table 3 Chlorophyll content of *Gracilaria* sp. (mg/g) on different maceration time.

Treatments (hours)	Average content of chlorophyll (mg/g)
A (0)	0.16 ^a ± 0.05
B (6)	0.19 ^a ± 0.05
C (12)	0.29 ^{ab} ± 0.07
D (18)	0.33 ^{ab} ± 0.07
E (24)	0.39 ^{bc} ± 0.08
F (30)	0.44 ^{bcd} ± 0.06
G (36)	0.54 ^{cde} ± 0.18
H (42)	0.61 ^{de} ± 0.13
I (48)	0.69 ^e ± 0.12

Description: different alphabet notation on the graphic shows significant differences among treatments (p<0.05).

Table 3 shows that longer maceration time also gave effect to chlorophyll content of *Gracilaria* sp. The highest chlorophyll content was obtained from treatment without maceration for 48 h, while the lowest chlorophyll content was obtained in treatment without maceration. Chlorophyll content in *Gracilaria* sp. is lower than phycoerytrin content. According to Veronica [18], chlorophyll content is relatively lower than phycoerytrin in Rhodophyta.

4. Conclusion

Different maceration time gave significantly different effect on phycoerytrin content and color brightness from *Gracilaria* sp. Treatment with 30 h of maceration time was the best treatment that obtained phycoerytrin as much as 0.98 mg/g and brightness value of 19.73 L*. reddish value of 7.23 , and yellowish value of 2.87 from *Gracilaria* sp.

5. References

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