Increasing β-carotene content of phytoplankton *Dunaliella salina* using different salinity media

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Increasing β-carotene content of phytoplankton *Dunaliella salina* using different salinity media

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**Abstract.** *Dunaliella salina* have got great attention in the nutritional, pharmaceutical and cosmetic companies because contain β-carotene. β-carotene functions as antioxidants and precursors of vitamin A and can treat tumors and cancer in humans. The content of β-carotene in *D. salina* can be increased by increasing salinity levels in the culture medium. The aim of this study was to determine whether increasing salinity may increase β-carotene content of phytoplankton *D. salina*. The research use data collection method with direct observation and then analyzed the result with descriptive method. The results showed that different salinity of media could influence β-carotene content of *D. salina*. The highest β-carotene content of *D. salina* was at treatment B (30 ppt) which equal to 2.312 mg/L on 10th day. The production of β-carotene in *D. salina* can be increased was other environmental stress treatments in the form of stress-temperature, light and nutrients using.

1. Introduction

*Dunaliella salina* is one of the most potent natural feeds as used feed additive and feed supplement in aquaculture [1]. Based on research conducted by abd EL-Baky [2], *D. salina* accumulates a high amount of carotenoids (12.6% of the dry weight) which comprising β-carotene (60.4%), α-carotene (17.7%), zeaxanthin (13.4%), lutein (4.6%) and cryptoxanthine (3.9%). Salinity is one of environmental stressors that can affect the increase of β-carotene content of *D. salina*. Based on the above discussion, this study was expected to see whether the difference of salinity concentration can influence the increase of β-carotene production in *D. salina*, so that the best salinity concentration can be identified to obtain the maximum amount of β-carotene in *D. salina*.

2. Methodology

2.1 Research method

The phytoplankton culture of *D. salina* was conducted in Education Laboratory of Faculty of Fishery and Marine, Airlangga University, Surabaya. Meanwhile, the measurement of β-carotene content was carried out at Pharmacy Testing, Airlangga University, Surabaya. The research data were collected through direct observation, which is an approach or technique to get primary data by observing research objects directly [3]. Data obtained from culture process and calculation of β-carotene content were then analyzed using descriptive method [4]. The results are presented in tables and figures.
2.2. Materials
The equipments used in this research were glass tube, aeration hose, aerator, reaction tube, reaction tube shelf, measuring cylinder, digital balance, dropper pipette, micro pipette, binocular microscope, haemocytometer, scissors, cuvet, handtally counter, spectrophotometer, culture shelf, refractometer, thermometer, pH pen, lux meter, autoclave, glass cover, vortex, centrifuge, dark plastic, styrofoam, 40 Watt lamp, and aluminum foil. The materials used in this research were D. salina seed and Walne medium (Brackish Water Cultivation Center (BBAP) Situbondo), clean seawater from Kenjeran Surabaya (30 ppt), aquades, petroleum ether (PE) alcohol, chlorine and Na-Thiosulfate.

2.3. Research procedure
2.3.1. Equipment and material preparation
The heat-resistant equipment was sterilized with autoclave at 121 °C for 15 minutes. Heat-resistant apparatus was also sterilized by immersion in a chlorine solution and neutralized with Na-Thiosulfate. Sterilization was also done on the culture medium and sea water. Before being sterilized, the salinity level of the sea water was measured using refractometer. Seawater that had been treated was further sterilized by giving a solution of chlorine and neutralized using a solution of Na-Thiosulfate [5].

2.3.2. Culture media for Dunaliella salina
The culture of D. salina in this study was incubated at 25-30°C with light intensity of 1200 lux using 40 Watt lamps with a dark period of 12 hours and light 12 hours [6].

2.3.3. Seedling Dunaliella salina
Planting of D. salina seeds was done after calculating stock density by taking samples of plankton from the stock medium and counting under a microscope with haemocytometer to the sample population calculation. Seed D. salina is fed to a medium with a density of 105 cells/ml [7].

2.3.4. Harvest of Dunaliella salina
The harvesting of phytoplankton D. salina was done partially on the 2nd, 4th, 6th, 8th, 10th, 12th and 14th days [6]. The harvesting process of D. salina was done up to the stationary phase. Harvesting D. salina was carried out by putting 10 ml samples into the reaction tube. It was then stored in the refrigerator at 5°C-15°C [5]. After that, the β-carotene content in D. salina was calculated.

2.3.5. Extraction of β-carotene from Dunaliella salina
Extraction of β-carotene content was carried out by taking 1 ml of D. salina yield using pipette 8 ml of distilled water was added and homogenized using vortex. The mixture (2 ml) was added to the test tube contained 2 ml of 96 % alcohol and 10 ml of petroleum ether (PE). They were then homogenized with vortex and centrifuged for 5 min. After centrifugation, two layers were formed was the lower supernatant and petroleum ether layer (PE) at the top. The petroleum ether (PE) layer formed was separated and marked as layer I while the remainder was added again with 10 ml petroleum ether (PE). The mixture was homogenized again for 2 minutes using a vortex and then centrifuged for 5 minutes. The petroleum ether (PE) layer formed was separated again and marked as layer II. Layers I and II were homogenized 2 ml of each layer was then taken and read on spectrophotometers with wavelengths of 480 nm, 645 nm and 663 nm [8].

2.3.6. β-carotene count on Dunaliella salina
The calculation of β-carotene content in phytoplankton D. salina was determined by using spectrophotometer. The extraction results of the petroleum ether (PE) layer formed subsequently were incorporated into the cuvet and read using a spectrophotometer with blanks ie petroleum ether (PE) solution [9]. The calculation formula of β-carotene content in D. salina [10] is as follows:

\[
β\text{-carotene (mg/L) = } \frac{(Abs 453 – Abs 665) x 3.657 x 3 x X}{3.91}
\]
Note:
Abs 453: Absorbance at 453 nm
Abs 665: Absorbance at 663 nm
3,657: Calibration factor on β-carotene content analysis
X: Dilution factor for measuring absorbance in spectrophotometer

3. Result and discussion
The result of the calculation of β-carotene content was obtained by using the specimen. Samples were taken on the 2nd, 4th, 6th, 8th, 10th, 12th and 14th day.

![Figure 1](image-url)  
**Figure 1.** Average of β-carotene content of *D. salina* cultured with different salinity.

Based on the average graph of β-carotene content (figure 1), it can be seen that the highest β-carotene content was in treatment B (30 ppt) i.e: on the 10th day of 2.312 mg/L, while treatment A (20 ppt) and C treatment (40 ppt) occurred on day 8 of 1.275 mg/L and 1.2648 mg/L and treatment D (50 ppt) of the highest β-carotene content occurred on the 6th day of 0.5525 mg/L.

Salinity is one of environmental stressors that affects the increase of β-carotene accumulation in *D. Salina*. Higher salinity (hypertonic) may cause shrinkage of cells and lower salinity (hypotonic) may result in cell swelling [6]. According to Ramos *et al.* [11], *D. salina* has two ways to defend itself from the salinity stress, that is by producing glycerol in the short term and producing carotenoids in the long term.

The results of the research show that the highest β-carotene content in *D. salina* was found in treatment B (30 ppt), that was 2.312 mg/L on the 10th day, while the β-carotene content in normal treatment was A (20 ppt), having the highest amount of 1.275 mg/L obtained on day 8. This shows that salinity can increase the β-carotene content in *D. salina*. It was proven that the content of β-carotene in treatment B (30 ppt) is higher than that of treatment A (20 ppt) as control or normal condition.

According to Shariati and Hadi [12], the content of β-carotene in *D. salina* can be increased with environmental stress of one salinity; the higher the salinity given, the higher the β-carotene content. However, in the C treatment (40 ppt) and D treatment (50 ppt), there were noticeable decreases in the total β-carotene content of 1.2648 mg/L and 0.5525 mg/L obtained. It is suspected that the *D. salina* microalgae have tolerance limits to the salinity stress given, so that too high salinity stress conditions can affect the decrease of β-carotene content in *D. salina* due to plasmolysis. The growth of *D. salina* is calculated daily for 14 days of maintenance by means of a haemocytometer device.
All treatments had lag phase on day 1. According to Ayustama and Sari [13], in the phase of the lag, microalga cell was still in the adaptation stage as an effort to adjust itself to the changing conditions of the initial media environment to the new media. The exponential phase occurred after the lag phase i.e.: on the 2nd day until the 7th day (for treatment A and C) and the 2nd day until day 9 (for treatment B) and the 2nd day until the 5th day (for treatment D), Dunaliella salina in this exponential phase grew rapidly.

The stationary phase occurred at day 8 (treatment A and C), the 10th (treatment B) and the 6th (treatment D). This phase occurred because the nutrients in the media had been greatly reduced so that it was insufficient for growth and cell division [14]. The phase of culture death occurred after the stationary phase at each treatment until the 14th day of maintenance. This phase is characterized by the color change of the culture water, the froth surfaces of the media culture and faded colors and clumps of algae cells that settle based on culture [13].

Based on the research, it was found that the highest density was obtained at treatment B (30 ppt) (figure 2), which was 6154.3 x 104 cells/ml on day 10, while treatment A (20 ppt) and C (40 ppt) had peak density on day to-8 with respective densities of 1555.85 x 104 cells/ml and 1523.1 x104 cells/ml and treatment D (50 ppt) which experienced peak density at day 6 of 672.5 x 104 cells/ml.

Figure 2 also showed that at 30 ppt salinity, the amount of D. salina cell density increased compared to other treatments (20 ppt, 40 ppt and 50 ppt) because at salinity of 30 ppt, this D. salina microalgae could still tolerate salinity stress given so that the growth tended to be normal as an adaptation system in the face of salinity stress D. salina forms β-carotene. Meanwhile, the amount of density of D. salina cells in salinity 40 ppt and 50 ppt tended to decrease because D. salina microalgae have plasmolysis or cell rupture due to too high osmotic pressure which cannot be tolerated. It affects cell death so that the production of β-carotene also decreased.

Water quality measurements were performed daily in the morning and afternoon during the maintenance period. Water quality parameters measured were temperature, pH, salinity, and dissolved oxygen (DO) (table 1).

Table 1. Water Quality Range during Maintenance Period of D. Salina.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Satuan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>28,2-30,8</td>
<td>ºC</td>
</tr>
<tr>
<td>pH</td>
<td>7,5-8,6</td>
<td>-</td>
</tr>
<tr>
<td>Salinity</td>
<td>20-50</td>
<td>ppt</td>
</tr>
<tr>
<td>\textit{Dissolved Oxygen (DO)}</td>
<td>6,3-6,9</td>
<td>mg/L</td>
</tr>
</tbody>
</table>

Based on the research results, the average temperature data ranged from 28.2 to 30.8 ºC. The optimum temperature range can be regarded as being suitable for D. salina environment. was between 1 ºC-30ºC [15]. The mean pH value in the study ranged from 7.5 to 8.6. Based on these results, it can
be assumed that the pH value is under normal conditions. According to Tafreshi and Shariati [16], *D. salina* has a high pH tolerance of 0-11 and the optimum pH for growth culture of *D. salina* microalgae is between 9-11. The mean DO values in the study ranged from 6.3-6.9 mg/L. The range of DO can be categorized suitable for the environment of *D. salina*. The optimum Dissolved Oxygen (DO) value for the growth of *D. salina* is between 6-8 mg/L [16].

4. Conclusions

Differences in salinity concentration can increase the content of β-carotene in *D. salina*. The best salinity to obtain the highest β-carotene content in *D. salina* was 30 ppt with the amount of β-carotene of 2.312 mg/L on day 10.

5. References

[1] Zainuri M H P Kusumaningrum and E Kusdiyantini 2006 Microbiological and Ecophysiological Characterization of Green Algae *Dunaliella* sp. for Improvement of Carotenoid Production. Faculty of Fisheries and Marine Sciences. Diponegoro University pp 1-1

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