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Research article

Changing of Chlorophyll Contents in *Caulerpa lentillifera* After Five-Day Harvest

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Abstract: *Caulerpa lentillifera* is a marine macroalgae widely consumed in Asia Pacific, including Thailand. Quality and freshness are the main criteria for consumers. Chlorophyll is an important fundamental pigmentation and acts as green in *C. lentillifera.* It can be variable according to light and growth, and then it can indicate the quality of *C. lentillifera* products. This research aimed to observe changing chlorophyll contents in C. lentillifera after harvest during five days from two different months- May and June and two different ponds-ponds 1 and 2 by chlorophyll extraction and measurements of chlorophyll fluorescence. The result showed that the contents of total chlorophyll and chlorophyll A differed between months, but the pattern did not significantly change even in different months and ponds within five days after harvest.

Keywords: *Caulerpa lentillifera*; Chlorophyll; Harvesting

1. Introduction

C. lentillifera is a famous culture species in the Indo-Pacific [1,2]. Because of its grape shape [3], the common name of this species is sea grapes or green caviar. The upright *C. lentillifera* thallus can be up to 10 centimeters long branch. Ramuli has a spherical tip about 1-3 cm in diameter and ramuli are densely clustered on each algae component [4]. *C. lentillifera* grows on rocks or the shallow water sandy ground near the coral reef. It can be found in muddy sand and can be acclimatized to grow well in ponds but cannot tolerate fresh water. It is distributed in tropical and subtropical regions. It is now commonly eaten in Asia-Pacific countries such as Japan, Vietnam, the Philippines, Malaysia, Indonesia, and Thailand [2, 5]. It has become about 1 ton/month in demand in the Thai market. *C. lentillifera* farms are widely along the Gulf of Thailand coast, rich in water nutrients and sunlight. The harvest in those areas is either from natural or ponds [4].

Much research shows that C. lentillifera is one of the functional food causes of rich minerals, essential amino acids, and fiber [1, 6-9]. These may support why this species became in higher demand in the market besides its shape. However, quality and freshness are important considerations for consumers as fresh purchases. Therefore, seaweed pigments can observe one of the quality criteria.

Green seaweed contains an important basic pigment that plays an important role in capturing sunlight for photosynthesis. The basic pigment group is chlorophyll; chlorophyll A is the primary pigmentation. Because it changes with the amount of light intensity, it can also be an indicator of the quality of seaweed [10]. This was the reason for studying the changes in the amount of pigment in *C. lentillifera* after harvesting for sale. The rate of change of chlorophyll content was examined when the algae were harvested for a longer time according to transportation from ponds to the marketplaces. To determine whether seaweed quality after harvest will likely decrease or increase. Therefore, this study aimed to study the chlorophyll change pattern within five days of culture *C. lentillifera* after harvest. The temporal and spatial variation of the chlorophyll change were compared to the environmental condition.

2. Materials and Methods

2.1 Study site and sampling

Samplings were carried out at open-pond cultures (Figure 1) in Ban Laem, where the commercial and original spread out of the fresh Caulerpa around Phetchaburi province and other markets. The test was set to investigate the pattern of chlorophyll quantity change in temporal (May to June) and spatial variation (ponds 1 and 2) during five days of harvest. Five days were settled as more time was spent transporting from pond to sold. The experiment started at the month of growth peak in May and June 2017. *Caulerpa* samples were randomly collected from the pond, placed in a box, and transferred to the laboratory (Figure 2). All specimens were kept in the dark container at room temperature until the day of the test. Specimens were sub-sampled for a series of each-day experiments.

Figure 1. A bottom planting culture pond at Ban Lad in Phetchaburi province.

2.2 Chlorophyll extraction

Specimens (approximately 1 gram) were cleaned from the sediment and then went through the steps of the chlorophyll extraction process. Fresh *Caulerpa* samples were extracted with 80% acetone. The modified chlorophyll extraction protocol was followed by Arnon [11] and Hui et al. [12]. Briefly, specimens were chopped into small pieces and digested in 80% acetone in a cool and dark condition for 5 minutes, then centrifuged at 4,000 *g* for 15 minutes. The supernatant was taken to the cuvette for light absorbance at different wavelengths via spectrophotometer. The total chlorophyll and chlorophyll A were calculated by the equation (1-2) below [12].

$$\text{Total Chlorophyll} = 8.02A_{663} + 20.21A_{664}, \tag{1}$$

$$\text{Chlorophyll A} = 11.85A_{664} - 1.54A_{647} - 0.08A_{630} \tag{2}$$

Figure 2. Fresh *Caulerpa* samples from a culture pond in Phetchaburi province.

2.3 Statistical analysis

Five replications were done throughout all experiments. Two-way ANOVA was employed on the total chlorophyll and chlorophyll A amount on different days, comparing months and ponds. All data were checked for the normal distribution as the criteria. All analyses were carried out with SPSS version 16.0 (SPSS Inc., USA)

3. Results and Discussion

Total chlorophyll and chlorophyll A content were used to determine the change in *Caulerpa* pigmentation. They showed parallel change, as expected, for the main pigment accumulation for green seaweed. The difference in total chlorophyll and chlorophyll A showed a similar trend of wavelength absorbance. Chlorophyll A is the main pigmentation of the green algae [13]. Therefore, changing the main pigmentation would affect the total chlorophyll in cell accumulation [14]. Also, A consistent change in total chlorophyll and chlorophyll A values should give a similar result in similar values when the experiment was done at the same time [15].

3.1. Comparing chlorophyll contents between months of study along five days

By comparing the changes in chlorophyll contents after sampling, it was found that total chlorophyll and chlorophyll A content were changed in similar patterns in both months studied, with fluctuation during the test in May (Figure 3). Chlorophyll contents showed higher in May than in June. In May, the significant difference of both concentrations was found during five days when $p < 0.05$. The high variation was along days 2-4 by a range of 0.68 ± 0.1 mg/l to 0.98 ± 0.2 mg/l for total chlorophyll and a range of 0.34 ± 0.05 mg/l to 0.48 ± 0.1 mg/l for chlorophyll A. It caused a significant difference in chlorophyll content during five days in May.

However, the content between the first day (0.39 ± 0.24 mg/l of total chlorophyll and 0.21 ± 0.12 mg/l of chlorophyll A) and the last day (0.46 ± 0.2 mg/L of total chlorophyll 0.27 ± 0.09 mg/l of chlorophyll A) of the test were not slightly difference. In other words, there was no significant change in June when $p > 0.05$ for both total chlorophyll and chlorophyll A during five days. The range of total chlorophyll was from 0.39 ± 0.1 mg/l to 0.68 ± 0.25 mg/l and 0.18 ± 0.08 mg/l to 0.32 ± 0.09 mg/l for chlorophyll A.

There was a temporal variation in the total chlorophyll and chlorophyll A between different months of sampling and along the day of study. The high fluctuation of those contents occurred in May, which caused the significant difference between months of study when $p < 0.05$. May showed a higher range of both contents than June, except for the first day of total chlorophyll content. Total chlorophyll and chlorophyll A on the first day were 0.39 ± 0.25 mg/l and 0.21 ± 0.12 mg/l in May and 0.68 ± 0.25 mg/l and 0.32 ± 0.09 mg/l in June, respectively. Otherwise, both contents had no significant differences when $p > 0.05$ on the first day of the study. The significant differences between months showed in days 2 and 3 for total chlorophyll when $p < 0.01$ (Figure 3A) and in days 2, 3, and 4 for chlorophyll A when $p < 0.05$ (Figure 3B).

The chlorophyll concentration was in May rather than June at the start date. This refers to the temporal variation in open culture, which can be affected by the multifactor of environment such as light intensity, temperature, and rainfall. Higher light intensity in May might affect the cell's biological mechanism and raise the chlorophyll content. This evidence could be confirmed by previous research in *Spirulina subsalsa.* The result showed that the decrease of the photosynthetic rate at high light energy was accompanied by increased antioxidant network response, such as carotenoids, total polyphenols, and antioxidant capacity. [16]. The chemical accumulation and function can cause environmental differences that influence seaweed growth [17]. Even though the culture has been manipulated throughout the year in Thailand, *C. lentilifera* grows in the dry season, which starts in February and peaks in May and June, during which this study began. The result of this study referred only to the condition of healthy specimens observed in May and June.

3.2 Comparing chlorophyll contents between ponds of study along five days

Sampling was done in the same months but in different ponds-pond 1 and 2. It indicated the culture spatial variation in total chlorophyll and chlorophyll A of *C. lentilifera.* The total chlorophyll and chlorophyll A changes were not significantly different over five days or between observing ponds. The total chlorophyll varied on the first study day when $p < 0.05$ (Figure 4). The average of total chlorophyll and chlorophyll A for five days were 0.48 ± 0.08 mg/l and 0.53 ± 0.19 mg/l for pond 1 and 0.23 ± 0.08 mg/l and 0.26 ± 0.08 mg/l for pond 2. Typically, the physiological activity of seaweed deteriorates after harvesting. That also causes quantity and quality loss in seaweed. In other words, they arose from changes in both physical and chemical components. Its expression would be found as outward shape, taste, and texture change [18]. These transformations would gradually occur over time after harvest. The constituent pigments, such as chlorophyll, will decompose over time when seaweed has been harvested. However, total chlorophyll and chlorophyll A were not significantly different change during five days after harvest in this study. The result showed that the amounts of chlorophyll A and total chlorophyll were not much different from the five-day study. Chlorophyll may be degraded to some extent, as indicated in the concentration fluctuation. Despite this, it is not so much that it can be a significant difference experiment. Therefore, total chlorophyll and chlorophyll A can remain in the algae tissue within five days without losing all these pigments.

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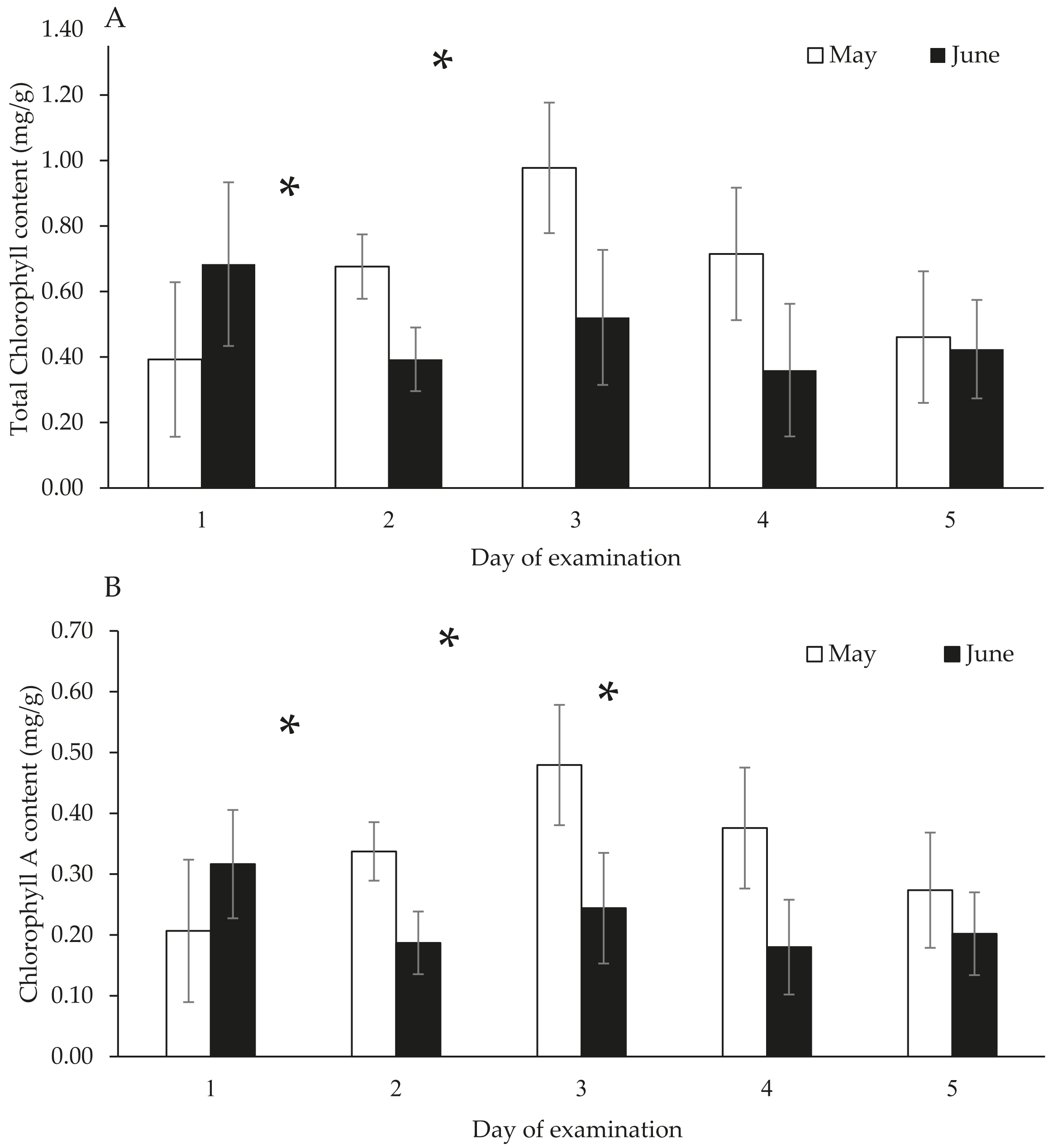

Figure 3. Temporal variation of the total chlorophyll (A) and chlorophyll A (B) contents between May and June during five days. Data represent mean ± SD. Asterisk showed a significant difference between the two months of study.

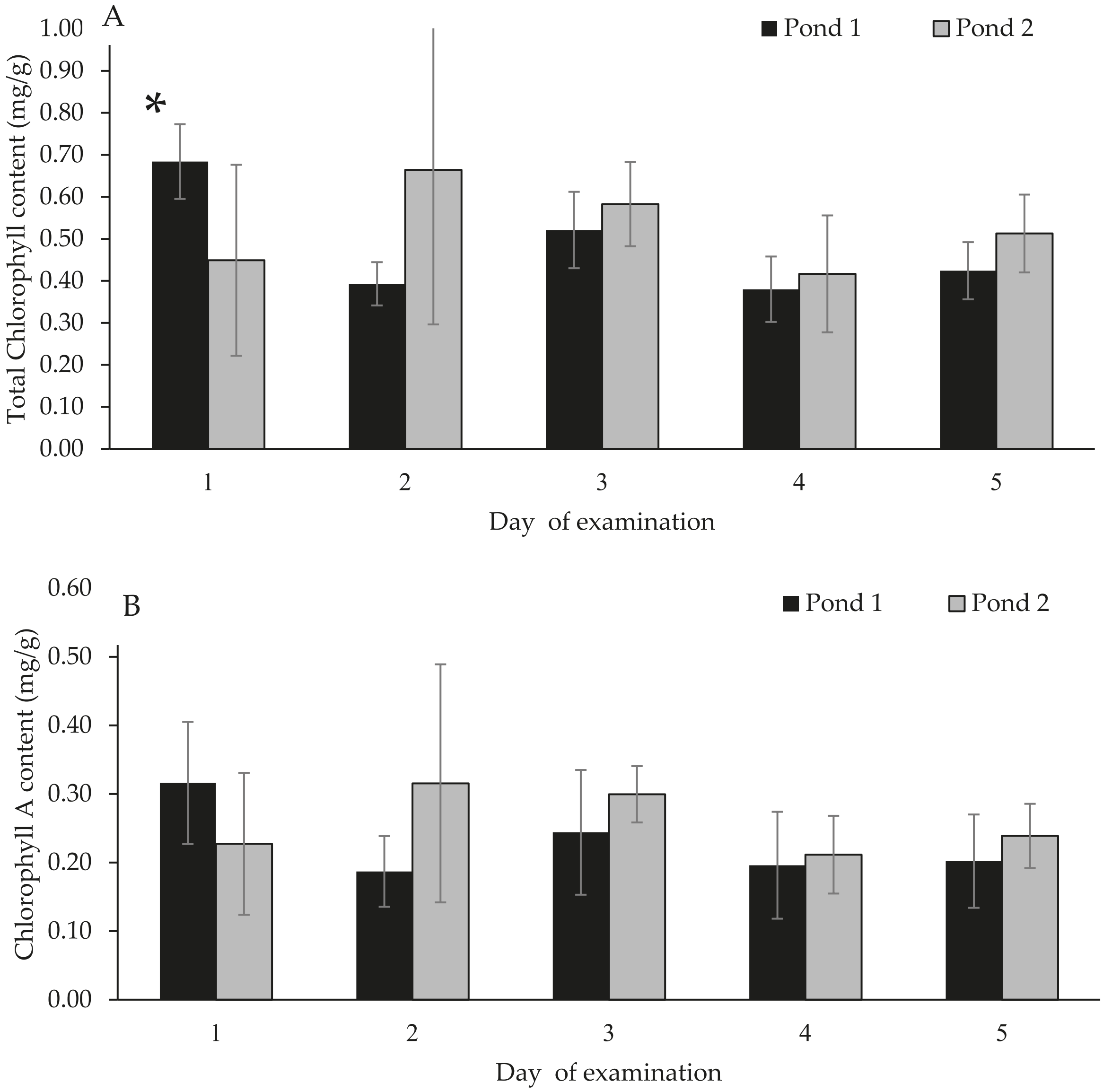

Figure 4. Spatial variation of the total chlorophyll (A) and chlorophyll A (B) contents between Ponds 1 and 2 during five days. Data represent mean ± SD. Asterisk showed a significant difference between the two months of study.

4. Conclusions

In conclusion, the chlorophyll accumulation did not change much in five days after harvest. The fluctuation according to spatial and temporal variation can be observed in *C. lentilifera.*

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Conflicts of Interest: The authors declare no conflict of interest.

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