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Production of β-cryptoxanthin at Different Artificial Light Spectra by Three Strains of Microalgae

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Abstract

Microalgae have significant potential for β-cryptoxanthin production. This study aimed to evaluate the effects of white (445, 544 nm), blue (465 nm), and red (660 nm) light-emitting diodes (LEDs) on biomass accumulation, total carotenoid content, and β-cryptoxanthin production in three strains of microalgae: *Scenedesmus obliquus*, *Coelastrum morus*, and *Chlorococcum* sp. Biomass accumulation increased under blue and red LED cultivation, while red LED significantly enhanced carotenoid and β-cryptoxanthin accumulation. β-Cryptoxanthin content in *S. obliquus*, *C. morus*, and *Chlorococcum* sp. cultivated under red LED was 171.92 ± 10.42, 217.35 ± 9.17, and 256.27 ± 8.80 μg/g cell dry weight, respectively. These values represent a 29.43%–33.27% increase compared to cultivation under white and blue LEDs. The antioxidant activity of all microalgal extracts exceeded 85%. These findings highlight the potential of red LED lighting to enhance β-cryptoxanthin production in the investigated microalgae strains.

Keywords*: *β-Cryptoxanthin; Carotenoids; Microalgae; LED Artificial light; Bioactive compound; Antioxidant

1. Introduction

β-Cryptoxanthin (beta-cryptoxanthin) is a xanthophyll carotenoid with a chemical structure and bioactivity similar to those of β-carotene. However, β-cryptoxanthin presents a higher polarity than β-carotene due to its extra hydroxyl group at the third carbon atom of the β-ring. The conjugated double bonds (chromophore) in the β-cryptoxanthin structure not only facilitate light absorption but also contribute to both color and photoprotection in plants (Bunea et al., 2014; Takayanagi, & Mikai, 2014; Saini et al., 2015). Human serum contains six major carotenoids: lycopene, α-carotene, β-carotene, lutein, zeaxanthin, and β-cryptoxanthin. Three of these carotenoids, α-carotene, β-carotene, and β-cryptoxanthin are converted into vitamin A in the human body (Nakamura, & Sugiura, 2019; Promwong et al., 2023). β-Cryptoxanthin has gained particular interest in recent years due to its higher bioaccessibility and bioavailability than lycopene and β-carotene in human serum and tissues (Zhu et al., 2016). Several studies have demonstrated the potent antioxidant properties of β-cryptoxanthin, showing its bioactivity against cancer (Sugiura, 2015; Iskandar et al., 2016), diabetes (Montonen et al., 2004), and liver disorders (Yilmaz et al., 2015). Additionally, β-cryptoxanthin has been shown to ameliorate neuropathic pain (Park et al., 2017),

stimulate immunity, reduce blood pressure (Nakamura et al., 2016), and prevent bone loss (Sugiura et al., 2016).

Unlike other carotenoids, β-cryptoxanthin is found only in some fruits and vegetables. The highest concentration of β-cryptoxanthin was detected in butternut squash at 34.71 μg/g sample (Burri et al., 2016; Jiao et al., 2019). Commercially available natural β-cryptoxanthin is the product from the extraction of satsuma mandarin orange (18.00 μg/g sample). Interestingly, several microalgae, including *Scenedesmus obliquus*, *Spirulina maxima*, and *Chlorella vulgaris* could produce β-cryptoxanthin at 23.76, 20.13, and 15.05 μg/g dry weight, respectively (Abd El-Baky et al., 2003; Patias et al., 2017). The high growth rate and non-seasonal variation of microalgae led to available biomass all year round (Hiransuchalert et al., 2023). The higher β-cryptoxanthin content in microalgae, combined with their non-seasonal cultivation, makes them a promising source for β-cryptoxanthin production.

A protective defense against stressful environmental conditions such as high light intensity, temperature, salinity, and the limitation of nitrogen and phosphate could elevate carotenoid synthesis (Faraloni, & Torzillo, 2017). Stressing by highlight intensity is the best inducing technique to enhance carotenoid production by several microalgae species (Sun et al., 2018). Increasing the light intensity could enhance the production of lutein to 3.6 mg/L/day by *Desmodesmus* sp., astaxanthin to 11.5 mg/L/day by *Haematococcus pluvialis* and β-carotene at 3 g/L/ in 5 cultivation days by *Dunaliella salina* (Aflalo et al., 2007; Lamers et al., 2010; Xie et al., 2013; Wolf et al., 2021).

The study on the effect of blue and red light-emitting diode (LED) light irradiation on carotenoid metabolism in satsuma mandarin (*Citrus unshiu* Marc.) showed that the accumulation of β-cryptoxanthin was induced by red light, not by blue light (Ma et al., 2012). White, blue, and red LED were applied to enhance the production of biomass and carotenoids by microalgae in the two-stage culture (Ma et al., 2018; Jung et al., 2019). Three microalgal strains, *Scenedesmus obliquus*, *Coelastrum morus*, and *Chlorococcum* sp. were reported as high-potential strains for carotenoids production (Rauytanapanit et al., 2019; Laje et al., 2019) and *S. obliquus* showed high production of β-cryptoxanthin (Patias et al., 2017). To the best of our knowledge, the effects of LED spectra on β-cryptoxanthin production in these microalgal strains have not been previously reported.

2. Objective

This research aimed to investigate the effect of different LED wavelengths on β-cryptoxanthin production and total carotenoid content in these three strains. The information obtained could be used for algae cultivation to enhance the β-cryptoxanthin production, which can be further used for functional food applications.

3. Materials and Methods

3.1 Chemicals

Standards of β-cryptoxanthin (≥ 97%), potassium hydroxide (KOH) and sodium chloride (NaCl) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The compound, 2,2-diphentl-1-picreylhydrazyl (DPPH) was obtained from Sisco Research Laboratories Pvt. Ltd. (India). Trolox reagent was a product from Tedia (USA). Ammonium acetate, acetonitrile, methyl tert-butyl ether (MTBE), methanol (MeOH), petroleum ether, and diethyl ether were bought from LAB-SCAN (Gliwice, Poland). MeOH and MTBE were high-performance liquid chromatographic (HPLC) grade, while all other reagents and chemicals used were analytical grade.

3.2 Microalgae Strains and Culture Medium

Three strains of microalgae, *Scenedesmus obliquus* TISTR 8522, *Coelastrum morus* TISTR 8566 and *Chlorococcum* sp. TISTR 8266 were kindly provided by the Algae Library of Thailand Institute of Scientific and Technological Research (TISTR). Each strain was pre-cultured at 25ºC with 10 μmol/m^2/s of light intensity with 80 mL of BG11 liquid medium, pH 7 in a 250 mL Erlenmeyer flask. The cultivation was conducted in an incubator with reciprocal shaking at 120 rpm for 5 days before being transferred for further biomass production.

3.3 Biomass Production

Each microalgal strain was cultivated to produce cell biomass in a laboratory bottle (1000 mL) with 800 mL of BG11 medium, using a system consisting of a rubber stopper with a glass tube, an air stone, and a 0.22 μm polytetrafluoroethylene polymer (PTFE) filter. The cultivation was carried out with aeration at 600 mL/min for 14 days with 50 μmol/m^2/s of fluorescent cool white light, a photoperiod of 12:12 (dark: light cycle illumination) at 25ºC.

3.4 Identification of β-cryptoxanthin

Carotenoid components, including β-cryptoxanthin, were thoroughly extracted from each

freeze-dried sample (0.2 g) using a mortar and pestle with acetone and methanol. The supernatant was then centrifuged at 9,000 g, 10ºC, for 15 minutes, until it became colorless (Mandelli et al., 2012). The extract was filtered through a 0.22 μm polyethylene membrane and concentrated using a vacuum rotary evaporator (BUCHI R-114, Fawil, Switzerland) at 30 °C. The concentrated extract was further suspended in a mixture of petroleum ether/diethyl ether (1:1 v/v), and saponified with 10% (w/v) methanolic KOH at room temperature for 16 h. Alkali in the sample was removed by washing with 10% (w/v) sodium chloride.

Identification of β-cryptoxanthin in the algal cells was performed by comparison with a β-cryptoxanthin standard. Dionex Ultimate 3000 RSLC system liquid chromatography-high resolution mass spectrometry (LC-HRMS) installed with orbitrap mass analyzer system (QEXACTIVE plus, Thermo Fisher Scientific, Germany) was used for the analysis. Each compound was separated into samples by running with a Hypersil GOLD C18 column (100 × 2.1 mm, 1.9 μm Particle size, Thermo Fisher Scientific) at 37ºC. The carotenoid extract from the freeze-dried sample was suspended in methanol and filtered with a 0.22 μm polyethylene membrane before analysis by LC-HRMS. The mobile phase consisted of 3 mM ammonium acetate in methanol /water (70:30, v/v; mobile phase A) and 3 mM ammonium acetate in acetonitrile/diethyl ether (99:1, v/v; mobile phase B). The linear gradient was programmed as follows: 0:00 – 0:20 min 100% A, 3:50 – 15:50 min 100% B, and 15:75 – 20:00 min A. The flow rate was set as 0.5 mL/ min with an injection volume of 20 μL.

3.5 Effects of LED Light Wavelength on Biomass and Carotenoid Production

After the biomass production phase, all microalgal cells were transferred to a new medium at the same concentration for the experiment. The inoculum at 0.5 g/L was used for the cultivation of all microalgae strains. Light intensity for the growth of each microalgal strain was set at 50 μmol/m^2/s for white LED light (445, 544 nm), blue LED light (465 nm) and red LED light (660 nm). The temperature in the cultivation room was controlled at 25ºC. A control was conducted under the same operating conditions without LED light illumination. The microalgal growth was measured by a cell dry weight (CDW) method, measuring the cell biomass harvested at 8 days of cultivation. The cell biomass was harvested by centrifugation (Hermle Z206A, Germany) with 9,000 g for 15 min at 10ºC. The cell pellets were subsequently washed with distilled water before freeze-drying at -50ºC, 175 μmHg for 24 h in the freeze-dryer (LSCplus, Germany). Then, the freeze-dried samples were stored in a refrigerator at 4ºC until further analysis. All experiments were performed in triplicate.

3.6 Analysis

3.6.1 Dry Cell Weight

The biomass was measured by a dry cell weight (DCW) method (Ratha et al., 2016). Whatman GF/C filter papers (47 mm diameter, 1.2 μm pore size) were dried and weighed before using for the filtration for the algal biomass. The filter paper containing the sample was dried in a hot air oven for one hour at 80ºC and overnight at 60ºC. After 30 minutes in a vacuum desiccator, filter sheets were removed, and their weights were determined with an analytical balance. Drying and weighing were repeated until constant weights were achieved.

3.6.2 Total Carotenoid Content

The freeze-dried microalgae sample (0.2 g) was extracted using acetone and metanol. The carotenoid extract obtained from each microalga was mixed with petroleum ether and measured for its absorbance at 450 nm by a spectrophotometer. Total carotenoid content was calculated using the following formula:

$$\text{Carotenoid content } (\mu g/g) = [(A \times V \times 10^4)] / [A_{1cm}^{1\%} \times P]$$

where A = absorbance; V = total extract volume (mL); P = sample weight (g); $A_{1cm}^{1\%}$ =2592 (β-carotene extinction coefficient in petroleum ether). Finally, the carotenoid extract was flushed with N_2 and kept at -20ºC in the dark until further analysis (de Carvalho et al., 2012).

3.6.3 β-Cryptoxanthin Content

The analysis of β-cryptoxanthin content was performed by the high-performance liquid chromatography, HPLC (KNAUER Model AZURA, Berlin, Germany) with a system consisting of a pump (AZURA P 6.1 L), a diode array detector (AZURA DAD 2.1 L,) and a C30 YMC column 5 μm, 250 × 4.6 mm (YMC America, Inc.). The mobile phase was composed of methanol (mobile phase A) and methyl tert-butyl ether (mobile phase B) in a linear gradient. At the baseline, 25, 55, and 60 min, the ratios of the solvents were 95:5, 70:30, 35:65, and 95:5. The sample injection was 15 μL, with the flow rate of the mobile phase at 0.9 mL/min. The detection was performed at a wavelength of 450 nm. The chromatogram data

was processed using the Clarity Chrom software (KNAUER, Berlin, Germany).

3.6.4 DPPH Free Radical Scavenging

The scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to determine the antioxidant activity of the samples according to the methods described by Lim et al., (2007) and Rivero-Cruz et al., (2020) with slight modifications. The 20 µL of sample or standard solution was placed in a 96-wells microplate. Then 80 µL of 4 mM DPPH methanolic solution was added, and the plate was kept in the dark at ambient temperature for 30 min. Methanol was used as the blank. Trolox was used as the standard in the assay. The absorbance was measured at 540 nm using a microplate reader (Bio-Rad, iMark, USA). The percentage of radical scavenging activity was calculated using the following equation:

$$\% \text{ DPPH inhibition} = (A_{control} - A_{sample})/ A_{control}) \times 100$$

where $A_{control}$ is the mixture of methanol and DPPH solution; and A_{sample} is the mixture of sample extract and DPPH solution.

3.7 Statistical Analysis

The results were reported as the mean ± SD. IBM SPSS software (SPSS Inc.) version 28 for Windows, one-way analysis of variance (ANOVA) and post-hoc Duncan's test with $p < 0.05$ were used to determine the significance of the variables. A minimum of three replications was used for each experiment.

4. Results and Discussions

4.1 Biomass Production

The three microalgae strains were cultivated with light intensity 50 µmol/m^2/s of fluorescent cool white light. There was a drastic increase in biomass until approximately day 12, followed by stationary growth for *C. morus* and *Chlorococcum* sp. at day 14. The accumulation of the biomass production of *S. obliquus*, *C. morus*, and *Chlorococcum* sp. at 14 days was 2.67 ± 0.11 g/L, 2.35 ± 0.14 g/L, and 2.50 ± 0.11 g/L, respectively as presented in Figure 1. The biomass production among the three microalgae strains was not significantly different over 14-day cultivation period.

4.2 Identification of β-cryptoxanthin by LC-HRMS

The carotenoid extract from the three microalgal strains were analyzed by LC-HRMS and compared with the β-cryptoxanthin standard. The retention time (RT) of β-cryptoxanthin was 7.19 min. The fragment ions of beta-cryptoxanthin identity were molecular ion $[M]^+$ 552.4326, characteristic ion $[M-92]^+$ 460.3699 generated from carotenoids polyene (isoprene skeleton chain) losing a hydroxylated group and toluene (C_7H_8), identical ion m/z 119.0858 and elimination/cleavage of hydrocarbon at polyene (isoprene skeleton chain) of carotenoids (Korkerd et al., 2024). The extract also revealed the ion fragments corresponding to those fragment ions of the standard β-cryptoxanthin molecular ion ($[M]^+$) as shown in Figure 2.

The extracts from all three algae strains exhibited the same fragment patterns with the β-cryptoxanthin standard. Beta-cryptoxanthin contents from each algal strain calculated as µg/g cell dry weight are summarized in Table 1. The result showed that the β-cryptoxanthin can be produced by three microalgae, *S. obliquus*, *C. morus*, and *Chlorococcum* sp., and *Chlorococcum* sp. could produce higher β-cryptoxanthin content than *C. morus* and *S. obliquus*, respectively.

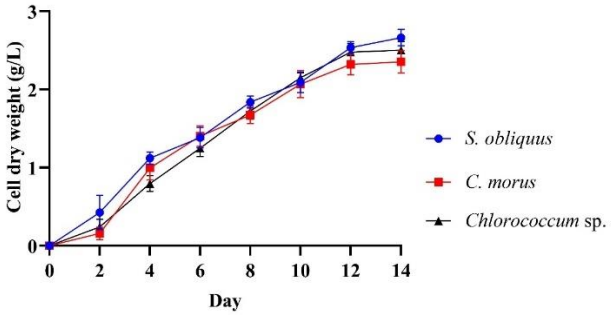

Figure 1 Biomass production of *S. obliquus*, *C. morus*, and *Chlorococcum* sp. with 50 µmol/m^2/s of fluorescent cool white light

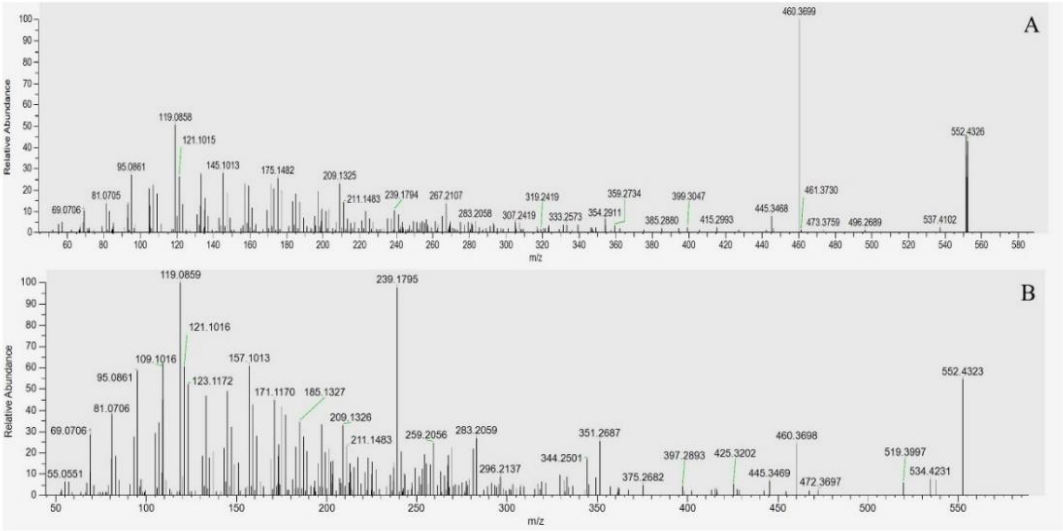

Figure 2 LC-HRMS spectra of β-cryptoxanthin from standard (A) and the extract from the *Chlorococcum* sp. (B)

Table 1 The β-cryptoxanthin production by algal strains grown under light at 50 μmol/m^2/s of fluorescent cool white light

Microalgal strain	β-Cryptoxanthin content (μg/g cell dry weight)
Scenedesmus obliquus	35.49 ± 3.51^c
Coelastrum morus	55.59 ± 4.97^b
Chlorococcum sp.	64.14 ± 2.54^a

Values are the average ± standard deviation of triplicates.

a, b, c, Different superscript letters in the same column correspond to significant differences ($p \leq 0.05$)

4.3 Effects of LED Light Wavelength on Biomass Production

As part of the photosynthesis process, microalgae need light as a source of energy to convert CO_2 and water into carbohydrates and oxygen. Photosynthesis was carried out in the presence of PAR (photosynthetically active radiation), comprising photons with a 400–700 nm wavelength and making up 43% of solar energy. Several main factors affecting photosynthesis are light intensity, spectral quality, and photoperiod (Htwe et al., 2023; Thimijan, & Heins, 1983; Lavens, & Sorgeloos, 1996). The growth of all algal strains with different LED light wavelengths was higher than that observed in the dark (control) condition. Even in the control condition without the light or heterotrophic condition, the biomass of all algal strains increased. Due to the microalgae growth in the new BG11 liquid medium at the first phase of cultivation, microalgae could grow in heterotrophic conditions by using the necessary organic sources in the BG11 medium. However, the use of blue LED (465 nm), and red LED (660 nm) enhance the growth of *C. morus* and *Chlorococcum* sp. more effectively than white LED (445, 554 nm). Illuminating the blue LED during cultivation significantly increased the biomass of *S. obliquus*, *C. morus*, and *Chlorococcum* sp. from 1.75 ± 0.13, 1.81 ± 0.14, and 1.91 ± 0.08 to 3.12 ± 0.23, 3.32 ± 0.16, and 3.30 ± 0.30 (g/L), which is about an increase of 56.09%, 54.52% and 57.88 respectively, compared to the control (Figure 3).

The biomass increase resulted from the maximum absorbance of each type of chlorophyll. Most plants and microalgae possess chlorophyll-a and chlorophyll-b. Microalgae can absorb different wavelengths of light based on their existing pigments. The ratio of chlorophyll-a to chlorophyll-b in green microalgae is 3:1. The two absorption peaks of chlorophyll-a are located at 430 nm (blue light) and 660 nm (red light), respectively. The absorption peaks of chlorophyll-b are located at 460 nm (blue light) and 630 nm (red light), respectively (Pattanaik et al., 2018). Red light and blue light can induce high growth rates by accelerating the cell division cycle, which results in an increased biomass of several microalgae strains from different evolutionary lines (Chen et al., 2010; Shu et al., 2012).

Similar results reported the LED influence on the algae *Porphyra leucosticte* and microalgae *Chlorella*

vulgaris by Korbee et al., (2005) and Al-amshawee, & Yunus (2019) showed that blue or red LED could stimulate their growth. Tran et al., (2015) reported that the ratio of red to blue light affected the growth and astaxanthin production of *Heamococccus lacustris*. At red to blue light ratios of 1:3, 2:2, and 3:1, the highest amounts of astaxanthin were 55.1, 50.3, and 36.3 mg/L, respectively. The maximum biomass of 1.48 g/L was obtained when the red, and blue were set at 1:3 illumination, followed by 1.36 g/L, 1.06 g/L, and 0.96 g/L when only the blue, red, and white light was used, respectively. The chlorophyll molecules in the microalgae can absorb a certain number of photons at blue or red wavelengths depending on their cellular structure, pigmentation, and chloroplast arrangement (Schulze et al., 2014).

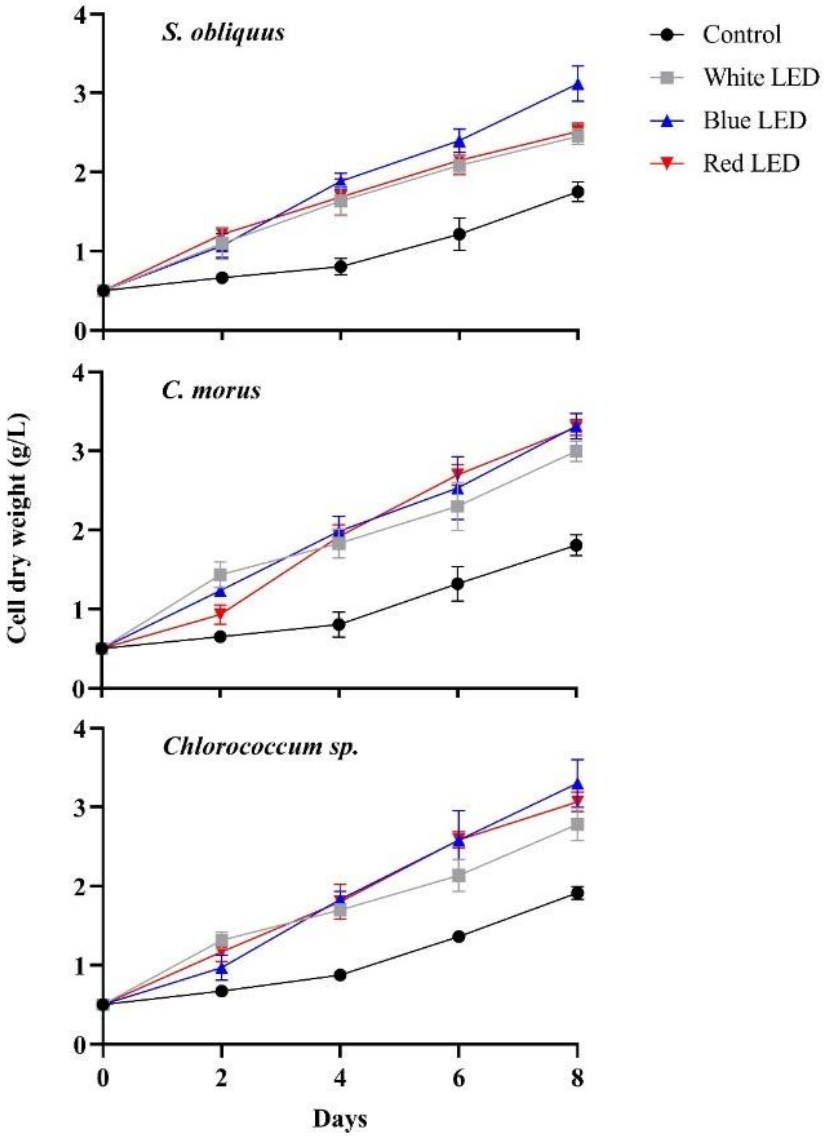

Figure 3 Cell dry weight obtained during the cultivation of each algal strain with different LED lights, while the control was the cultivation in the dark

4.4 Effects of LED Light Wavelength on β-cryptoxanthin Content and Total Carotenoid Content

The production of β-cryptoxanthin was significantly increased after the cultivation with LEDs light, especially with the use of red LED as shown in Figure 4A. Compared to the control, β-cryptoxanthin content in all algal strains was increased with the use of LEDs ($p \leq 0.05$). The β-cryptoxanthin content in each algae species did not differ significantly when cultivated under white or blue LED. However, β-cryptoxanthin produced by *Chlorococcum* sp. was much higher than that of *S. obliquus* and *C. morus*. The highest β-cryptoxanthin content obtained in *S. obliquus* (171.92 ± 10.42 μg/g CDW), *C. morus* (217.35 ± 9.17 μg/g CDW), and *Chlorococcum* sp. (256.27 ± 8.80 μg/g CDW), with the cultivation with red LED, presented an increase of 66.73%, 69.89%, and 70.57%, respectively, compared to the control.

The use of different LED wavelengths, especially the red LED, significantly affected the β-cryptoxanthin production as described above. Though total carotenoid content was significantly increased in all algae strains using all LED wavelengths ($p \leq 0.05$) compared to the control, a non-significant effect was obtained in each strain (Figure 4B). However, the use of LED significantly increased total carotenoids in *Chlorococcum* sp. than that of *S. obliquus*. *Chlorococcum* sp. cultivated with red LED had higher total carotenoid content than blue LED but was not significantly different from white LED at $p \leq 0.05$. Even grown in the dark condition, *C. morus* produced higher total carotenoid production than the other two strains. Total carotenoid content produced by *S. obliquus*, *C. morus*, and *Chlorococcum* sp. was increased with all spectrums of LED used. At the same time, the red LED showed the total carotenoid content at 2.37 ± 0.28 mg/g CDW, 3.35 ± 0.20 mg/g CDW, and 3.11 ± 0.21 mg/g CDW, respectively.

The cell composition or metabolism of microalgae can vary depending on light intensity during the cultivation, affecting light-related molecules such as chlorophyll and carotenoids (Danesi et al., 2011). Many microalgae can produce carotenoids, the well-known antioxidants which can protect photosynthetic organisms from excessive exposure to light by permitting triplet-triplet energy transfer from chlorophyll to carotenoid and by quenching reactive oxygen species (ROS) (Hashimoto et al., 2015; Erickson et al., 2015; Yokthongwattana et al., 2019). Xu, & Harvey (2019) reported that the content of secondary xanthophylls in microalgae, such as lutein and zeaxanthin, tended to increase with red LED light. The β-cryptoxanthin is one type of xanthophyll. Further study on the production of β-cryptoxanthin by *Scenedesmus obliquus*, *Coelastrum morus*, and *Chlorococcum* sp. with different intensities (50, 100, 200, and 300 μmol/m^2/s) of red LED was studied and reported that increasing the red LED could increase the β-cryptoxanthin (Chuechomsuk et al., 2025).

4.5 Effects of LED light Wavelength on the Antioxidant Capacity of the Microalgae Carotenoid Extract

Table 2 reveals the antioxidant capacity of the extract from microalgal strains cultivated at different conditions by measuring their ability to suppress DPPH radicals. The antioxidant activity of all extracts was higher than 85%. The antioxidant activity under white and blue LED did not differ significantly from the control in all microalgae strains. The use of the red LED showed that the antioxidant activity from the extract of *S. obliquus* and *Chlorococcum* sp. was significantly higher than the control. White LEDs showed results that were comparable to red LEDs but significantly higher than the control and blue LEDs for *C. morus*. The antioxidant activity under white and blue LED was not significantly different from the control in all microalgae strains, despite the results in Figure 4B showing that the total carotenoid content under white and blue LED was significantly higher than the control in all strains. This might be due to some bioactive compounds in the carotenoid extract from the three microalgae that have stronger antioxidant activity. For example, lycopene, zeaxanthin, lutein, and β-carotene (Johra et al., 2020). Therefore, In our further study, we plan to analyze the carotenoid profile to know more details.

Microalgae grown under red LED light tended to accumulate more carotenoids than those grown under other light conditions. Total carotenoid content significantly affects the antioxidant activity of the microalgae carotenoid extracts, resulting in different values of antioxidant inhibitions. The results were consistent with the total carotenoid content in the samples. According to Raposo et al., (2015), the antioxidant properties of carotenoids are significantly influenced by their structural characteristics. An increase in the proportion of DPPH inhibition caused by antioxidants may be due to the radicals' capacity to scavenge them through electron transfer or hydrogen donation (Limsitthichaikoon et al., 2024).

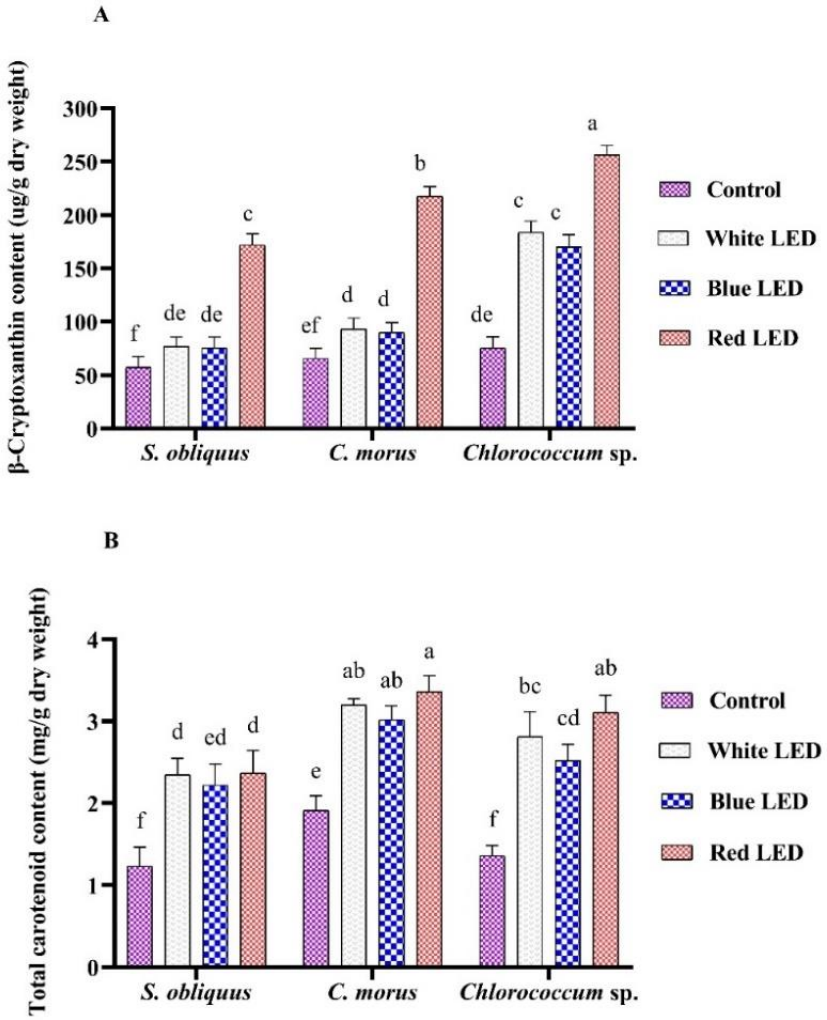

Figure 4 β-Cryptoxanthin production (A) and total carotenoids (B) by each microalgal strain with different LED lights at 50 μmol/m^2/s while the control was the cultivation in the dark. Different superscript letters in each bar mean significant differences ($p \leq 0.05$)

Table 2 Antioxidant activity of microalgae extracts with different LED lights for microalgae cultivation

Microalgae extract	DPPH radical scavenging activity (%)			
	Control	White LED	Blue LED	Red LED
S. obliquus	85.94 ± 0.21^{d}	86.43 ± 0.25^{bcd}	86.13 ± 0.21^{cd}	86.61 ± 0.26^{abc}
C. morus	86.49 ± 0.12^{bcd}	86.91 ± 0.48^{ab}	86.42 ± 0.37^{bcd}	87.20 ± 0.48^{a}
Chlorococcum sp.	86.35 ± 0.19^{bcd}	86.87 ± 0.45^{ab}	86.73 ± 0.45^{abc}	87.26 ± 0.41^{a}

Different superscript letters in each data value mean significant differences ($p \leq 0.05$)

5. Conclusion

In this study, the red LED light was more effective than blue LED, and white LED light, particularly in terms of β-cryptoxanthin production in *S. obliquus*, *C. morus*, and *Chlorococcum* sp. , which increased by 66.73%, 69.89%, and 70.57%, respectively, compared to the control. The carotenoid extract from microalgae also presented higher antioxidant properties, containing many conjugated double bonds. Moreover, as far as we are concerned,

this is the first report on the enhancement of β-cryptoxanthin production in *S. obliquus*, *C. morus*, and *Chlorococcum* sp. Therefore, to develop optimal conditions for β-cryptoxanthin production, it is necessary to study further the increase of red LED light more than 50 μmol/m^2/s or add some chemicals that affect the microalgae growth and increase β-cryptoxanthin content.

6. Acknowledgement

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