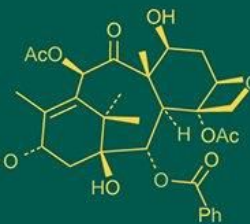
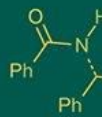


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Accumulation of carotenoid, lipid, phenolic and antioxidant capacity of microalgae *Dunaliella salina* ccap 19/18 cultivated under temperature stress regimes

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Abstract

Microalgae *Dunaliella salina* is considered a source of biologically active substances such as carotenoids specially beta-carotene, phenolics, and nutrients like lipids, glycerol, and carbohydrates under abiotic and biotic stress conditions. *Dunaliella salina* CCAP 19/18 was cultured two stages, the growth stage the microalgae cultivated in RM2 medium for 12 days to reach maximum cell density. The stress stage, after 12 days of cultivation, *Dunaliella salina* was exposed systematically to three distinct temperature conditions 10°C, 25°C (control), and 45°C. The analysis of carotenoid, lipid, phenolic compound and antioxidant capacity every 3 days at two different daily exposure durations: 3 and 6 hours. The results showed that the temperature stress cultivated conditions were induced the accumulation of biological active compounds rapidly, carotenoid, lipid and phenolic in *Dunaliella salina* cells. Specially, under the culture condition of 10°C for 6 hours/day, the accumulation of carotenoids (1.531 pg/cell), lipids (29.209 pg/cell), phenolics (1.197 pg GAE/cell) and antioxidant capacity (29.255 I% /cell) of microalgae *D. salina* CCAP 19/18 reached the highest concentration on day 12 after inhibition. However, under inhibitory culture conditions at 45°C, no microalgae cell survival was recorded after 3 days of inhibition. The temperature factor and stress regimes are known key markers for strategy to increase accumulation of carotenoid, lipid and phenolic compounds in cultivating microalgae *Dunaliella salina* in Viet Nam.

Keywords: *Dunaliella salina*, carotenoids, lipids, phenolics, antioxidant capacity, temperature.

Introduction

The halophilic green microalga *Dunaliella salina* is receiving increasing attention through the provision of highly biologically active substances under appropriate inhibition conditions, for application in biofuel production, wastewater treatment, fertilizer, medicine and food. China, India and the United States are the leading countries for the research contribution of *D. salina*, where the main focus of research is on carotenoid (specifically β -carotene) and lipid production [1]. β -carotene is a component of photosynthesis and is produced in lipid droplets in the thylakoids of chloroplasts under inhibitory conditions such as temperature, nutrients, light, salinity, pH to protect the photosynthesis apparatus and membrane integrity [2, 3].

Temperature is considered one of the factors that affects the growth process and composition of valuable substances in microalgae, the impact of temperature can cause changes in the synthesis of carotenoids or the composition of fatty acids. The studies have reported that increased temperature significantly reduces the amount of unsaturated fatty acids, affecting the maintenance and fluidity of algae cell membranes [4]. Research by Wu and colleagues (2016) investigated the effects of light, temperature and nutrition on the growth and pigment accumulation of three strains of *Dunaliella salina* KU XI, KU 10 and KU 31. The results showed that the optimal light and temperature for the growth of all three strains were 135,3 $\mu\text{mol.photons/m}^2/\text{s}$ and 22°C while the condition of 245,6 $\mu\text{mol.photons/m}^2/\text{s}$ and 22°C gave the highest amount of β -carotene. The optimal nutrient concentrations of KNO_3 , $\text{CO}(\text{NO}_2)_2$ and NaHCO_3 for the growth of the

three strains are 0,5 g/L, 0,36 g/L and 1,5 g/L, respectively 0 g/L, 0,12 g/L and 1,5 g/L gave the highest β -carotene content [5]. Another study by Reshma and colleagues (2021) aimed to evaluate the effects of pH, salinity, temperature, photoperiod and light intensity on the synthesis and accumulation of chlorophyll and carotenoids of microalga *D. salina* [6]. The results showed that growth, biomass, chlorophyll a, b, total carotenoids and β -carotene increased under optimized conditions of pH=7, salinity 37 PSU, temperature 23°C, photoperiod 12:12 and light intensity of 200 $\mu\text{m}^2/\text{s}$ when compared to normal conditions.

Antioxidants (enzymatic or non-enzymatic) are classified depending on their mode of activity as primary antioxidants (hydrogen or electrons donors) or secondary antioxidants (oxygen scavengers or chelating agents) [7]. Phenols are important natural compounds with antioxidant and other biological properties. Phenolic compounds effectively scavenge hydroxyl and peroxy radicals and prevent fats oxidation and have great advantages in food, chemistries, pharmaceutical and medical industries. Phenolic content of *D. salina* is increased at low temperatures. Low temperature along with light increase the oxidative stress by limiting photosynthetic enzymes thereby increasing the phenolics as photoprotective compounds [8]. Microalgae *D. salina* were cultivated at low temperature (13 °C) together with low light irradiation (20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) to enhance SOD, APX, MDHAR, DHAR activity compared to the unstressed controls, whilst cultivation at a very low temperature (5 °C) decreased CAT activity and less carotenoid and ascorbate contents at extremely low temperatures. SOD, APX, DHAR, activity were increased in *D. salina* (IR-1) when cells were grown for 2 days under high temperature (28 °C) under light irradiation ranging between 100-1200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ compared to a different strain (Gh-U) [7]. This study aimed to investigate the accumulation of carotenoid, lipid, phenolic compounds and antioxidant capacity of microalgae *D. salina* cultivated under the stress of temperature regimes. From the results, we can build strategies for culturing *D. salina* in another weather conditions in Viet Nam.

Materials and Methods

Materials

Microalgae strain *D. salina* CCAP 19/18 is cultured and stored at the laboratory of the Department of Biochemistry and Toxicology, Pharmacy faculty, Nguyen Tat Thanh University, grown in RM2 environment including salt field water (380‰) diluted with sea water to reach a salinity of 1.5M (90‰) then add nutrients: NPK 0,15 g/L, MgSO_4 1,86 g/L, EDTA 8,76 mg/L, FeCl_3 0,49 mg/L, MnCl_2 1,89 mg/L, pH = 7,5, at $25^\circ\text{C} \pm 2^\circ\text{C}$ with light intensity 70 $\mu\text{mol photons}/\text{m}^2/\text{s}$, light: dark cycle = 12:12 hours [9].

Methods

Determine cell density

Microalgae were counted directly using a Neubauer red blood cell counting chamber to determine cell density. Take 10 μL of algal solution fixed with Lugon solution (5% Iodine, 10% KI salt) into a red blood cell counting chamber with a depth of 0.1 mm, square area of 1 mm^2 and count cells under a 10X objective optical microscope, repeatability at least 3 times per sample [10].

Cell density per 1 mL was determined according to the formula:

$$D = \frac{n}{i} \times 10^4 \times \text{dilution factor}$$

N: total number of counted cells

I: counting area

D: cell density (cell/mL)

Total carotenoid content

Take 1 mL of algae solution and centrifuge it, discard the liquid and keep the algae. The algal residue was extracted with 3 mL of ethanol: hexane solvent mixture, then added to 15 mL of fanol and vortexed evenly. Continue adding 4 mL of hexane and 2 mL of distilled water to the fanol containing algae and then vortex until the color is gone. After centrifugation, the mixture will separate into 2 layers, the solvent layer containing the pigment will be absorbed and measured photometrically at wavelengths of 450 nm, 645 nm and 662 nm [12].

Total carotenoid content was determined using the formula [11]:

Total carotenoids ($\mu\text{g}/\text{mL}$): $A_{450} \times 25.2$

The content of chlorophyll a and b was calculated according to the formula [12]:

Chlorophyll a ($\mu\text{g}/\text{mL}$) = $11.75 (A_{662}) - 2.35 (A_{645})$

Chlorophyll b ($\mu\text{g}/\text{mL}$) = $18.61 (A_{645}) - 3.96 (A_{662})$

Total chlorophyll ($\mu\text{g}/\text{mL}$) = chlorophyll a + chlorophyll b

A_{645} is the absorbance at wavelength 645 nm

A_{662} is the absorbance at wavelength 662 nm

Determination of total lipid content by Sulfo-Phospho-Vanillin method

Prepare Phosphovanillin reagent: weigh 0.06 g of vanillin into a 50 mL volumetric flask covered with foil, add 2 mL of absolute ethanol and 8 mL of distilled water and shake well to dissolve. Then use phosphoric acid (H_3PO_4) to the mark and shake well [13].

Building a lipid standard curve equation: Canola Oil (total lipid content 100 g/100 g) was mixed in chloroform to a standard lipid concentration range of 10-140 $\mu\text{g}/\text{mL}$ and heated at 90°C for 10 minutes on the stove water bath to evaporate all chloroform. Next, add 2 mL of concentrated sulfuric acid into the test tube, vortex well and boil at 95°C for 10 minutes, then cool. Add 5 mL of phosphovanillin reagent and vortex until the mixture is homogeneous. The sample was measured photometrically at a wavelength of 530 nm [13]. From the results, proceed to build a linear regression equation $y=ax+b$ showing the correlation between optical density (OD, y) and lipid concentration (x, $\mu\text{g}/\text{mL}$).

Determination of total lipid content of microalga *D. salina* CCAP 19/18: take 1 mL of microalgae solution and centrifuge, discard the supernatant and keep the microalgal pellets. The pellet is extracted with 2 mL of concentrated sulfuric acid and followed by the above procedure. The sample was measured photometrically at a wavelength of 530 nm [14]. The total lipid content of the microalga *Dunaliella salina* was determined by a linear regression equation with (y) being the optical density (OD) value and (x) being the total lipid content ($\mu\text{g}/\text{mL}$).

Total lipids ($\mu\text{g}/\text{mL}$): $(A_{530}-b)/a$

a,b: constants in the lipid standard curve equation

y: OD value measured at wavelength A_{530}

x: total lipid content ($\mu\text{g}/\text{mL}$)

Determination of total phenolic content using Folin-Ciocalteu's phenol reagent

Building a phenolic standard curve equation: gallic acid is mixed in methanol to create a standard gallic acid concentration range from 1-50 mg/L. Take 500 µL of each concentration into a 2 mL eppendorf, add 500 µL of Folin-Ciocalteu's phenol vortex reagent evenly. After 5 minutes, slowly add 500 µL of 10% Na₂CO₃, mix well and incubate for 90 minutes. Centrifuge and measure photometrically at 750 nm. From the results, proceed to build a linear regression equation $y=ax+b$ showing the correlation between optical density (OD, y) and lipid concentration (x, µg/mL).

Determination of total phenolic content of microalgae *D. salina* CCAP 19/18: take 1 mL of microalgae solution separate, discard the liquid and keep the algae. The bite part was extracted with 1 mL of absolute methanol and incubated for 30 minutes in the dark and followed by the above procedure. After, centrifugation and photometric measurement at 750 nm [15]. The total phenolic content of the microalga *Dunaliella salina* was determined by a linear regression equation with (y) being the optical density (OD) value and (x) being the total phenolic content (µg/mL).

Total Phenolic (µg/ mL): $(A_{750}-b)/a$

a,b: constants in the phenolic standard curve equation

y: OD value measured at wavelength A_{750}

x: total phenolic content (µg/mL)

Determination of antioxidant capacity by DPPH test

Mix reagent: weigh 0.004 g of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) reagent into a 100 mL volumetric flask covered with foil, use absolute methanol to dissolve and make up to the mark.

Take 1 mL of microalgae solution and centrifuge, remove the supernatant and keep the pellet. The pellet was extracted with 1 mL of absolute ethanol, mixed well and incubated at 4°C for 4 hours. After, centrifuge the algal solution, pipet 500 µL of algal solution into a 2 mL eppendorf covered with foil, add 1 mL of DPPH reagent and incubate in the dark for about 30 minutes, optical density wavelength at 517 nm [16]. Antioxidant capacity (I%/mL) is calculated based on the ability of DPPH to inhibit free radicals according to the formula [17, 16]:

$$I\%/mL = \frac{A_{\text{blank sample}} - A_{\text{test sample}}}{A_{\text{blank sample}}} \times 100$$

I%: inhibition percentage

$A_{\text{blank sample}}$: absorbance of blank sample at wavelength 517 nm

$A_{\text{test sample}}$: absorbance of the test sample at wavelength 517 nm

The Experimental Design

Growth culture stage: microalgae strain *Dunaliella salina* CCAP 19/18 is cultured in RM2 medium with salinity of 1.5 M, initial cell density of 0.15×10^6 cells/mL, the cultural conditions: continuous aeration, temperature at $25^\circ\text{C} \pm 2^\circ\text{C}$, light intensity $70 \mu\text{mol photons/m}^2/\text{s}$, light: dark cycle = 12:12 hours. After 12 days of culture, cell density reached 1.2×10^6 cells/mL, the *D. salina* microalgae strain was transferred to temperature-stress culture conditions.

Inhibition culture phase: *D. salina* was cultured under three temperature conditions (10°C , 25°C , and $45^\circ\text{C} \pm 2^\circ\text{C}$) with

two daily exposure periods (3 and 6 hours), continuous aeration, light intensity $70 \mu\text{mol photons/m}^2/\text{s}$, light: dark cycle = 12:12 hours. Each treatment was repeated 3 times (Table 1).

Harvesting and analyzing microalgae: samples were collected once on day 12 before inhibition (Before stress) and days 3, 6, 9 and 12 during inhibition.

Table 1. Treatments were under temperature-inhibited conditions

Treatment	Symbol
Temperature 25°C	Control+T25
Temperature 10°C for 3 hours/day	T10+3h
Temperature 10°C for 6 hours/day	T10+6h
Temperature 45°C for 3 hours/day	T45+3h
Temperature 45°C for 6 hours/day	T45+6h

Data Analysis

Data were processed using Microsoft office Excel 2016 and analyzed by One Way ANOVA using SPSS 20.0 software with significance error $p \leq 0.05$. All data are presented as: Mean±Standard Error (SE)

Results and Discussion

Total Carotenoid Content

The total carotenoid content per volume of *D. salina* CCAP 19/18 showed a significant increase under temperature stress conditions, with the most pronounced increase observed at 10°C (Figure 1.1). Specifically, carotenoid content increased from 0.669 to $2.480 \mu\text{g/mL}$ over the 12-day period. In contrast, exposure to 45°C proved lethal to *D. salina*, resulting in complete cell mortality within 3 days of temperature treatment, as evidenced by microscopic examination and zero cell viability. The highest carotenoid concentration was observed in the T10+3h treatment, reaching $2.610 \pm 0.013 \mu\text{g/mL}$ on day 12, which was significantly higher than other treatments ($p \leq 0.05$). There was no significant difference compared to the control treatment Control+T25 ($2.480 \mu\text{g/mL}$) on day 12 ($p=0.976$) and higher than the control treatment T10+6h on day 12 ($p \leq 0.05$) (Figure 1.1). Total carotenoid content (pg/cell) of CCAP 19/18 reached the highest value in the T10+6h treatment (1.531 pg/cell) higher than most days after temperature inhibition ($p \leq 0.05$) (Figure 1.1). Similarly, the carotenoid/chlorophyll ratio reached the highest value in the treatment T10+6h after temperature inhibition ($p \leq 0.05$), especially on day 9 after inhibition, the carotenoid/chlorophyll ratio reached the highest (5.718). (Figure 1.2). Thus, under the cold temperature inhibitory culture condition of 10°C for 6 hours/day, *Dunaliella salina* CCAP 19/18 gives a higher carotenoid content than the two control culture conditions (Control+T25) and inhibit T10+3h.

According to Orset and Young, 2002, the main factor in stimulating the synthesis of carotene was determined to be exposure to lower than optimum temperatures for microalgal *Dunaliella salina* growth. A 7.5-fold increase in the levels of α -carotene was observed when the temperature was decreased from 34 to 17°C , whilst levels of β -carotene were unaltered [9, 18]. Potijun and colleagues (2021) studied *Chlamydomonas Reinhardtii* and found that there was an increase in carotenoid content under the influence of cold temperature conditions [19]. Meanwhile, under inhibitory culture conditions of 45°C for 3 and 6 hours a day, *D. salina* CCAP 19/18 does not grow. At this temperature, the cell

membrane of CCAP 19/18 ruptures. In addition, cells tend to stick together in clusters and are unable to survive the inhibition process. Research by Ras and colleagues (2013) shows that when temperature conditions exceed the optimal

threshold, the effects are more harmful than low temperatures, negatively impacting the decline in growth rate as well as causing increase the rate and death rate of microalgae ^[20].

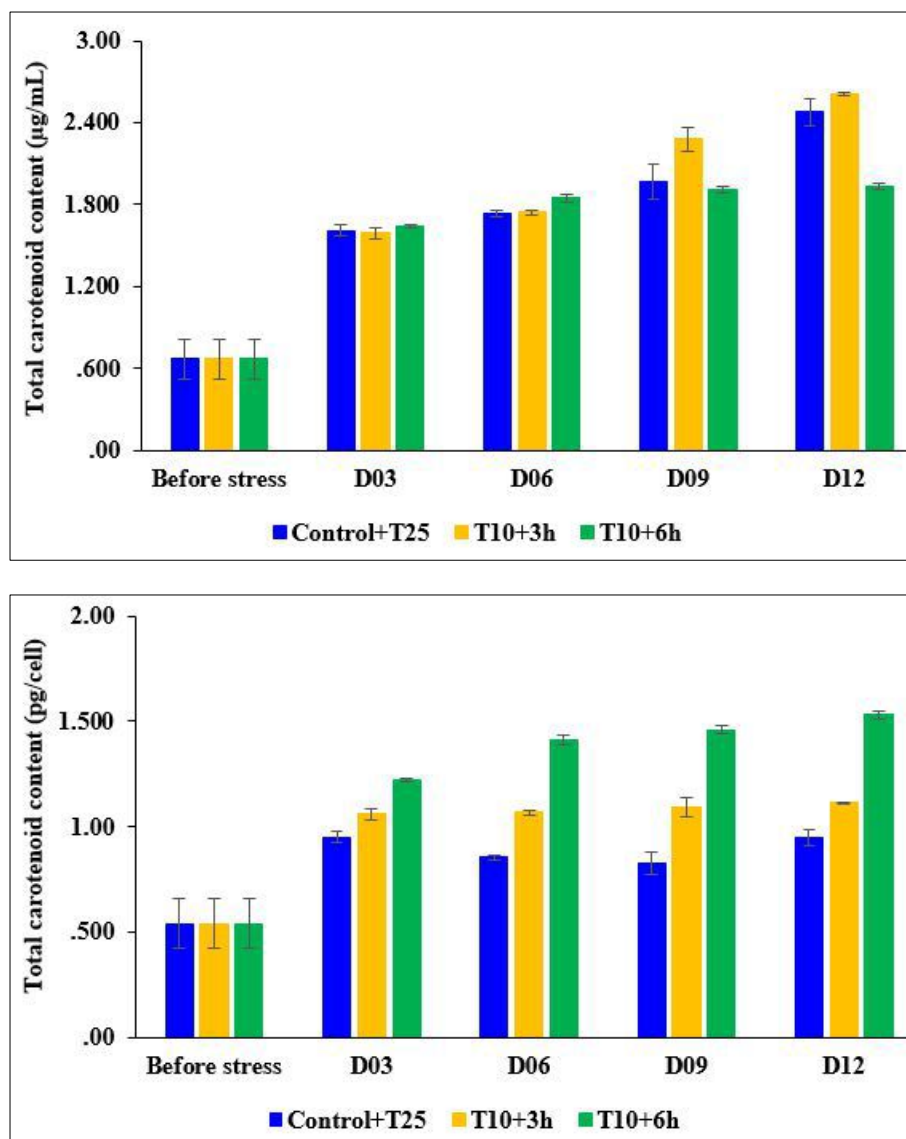


Fig 1.1: Total carotenoid content (µg/mL) and (pg/cell) of *D. salina* CCAP 19/18 under different culture conditions temperature inhibition

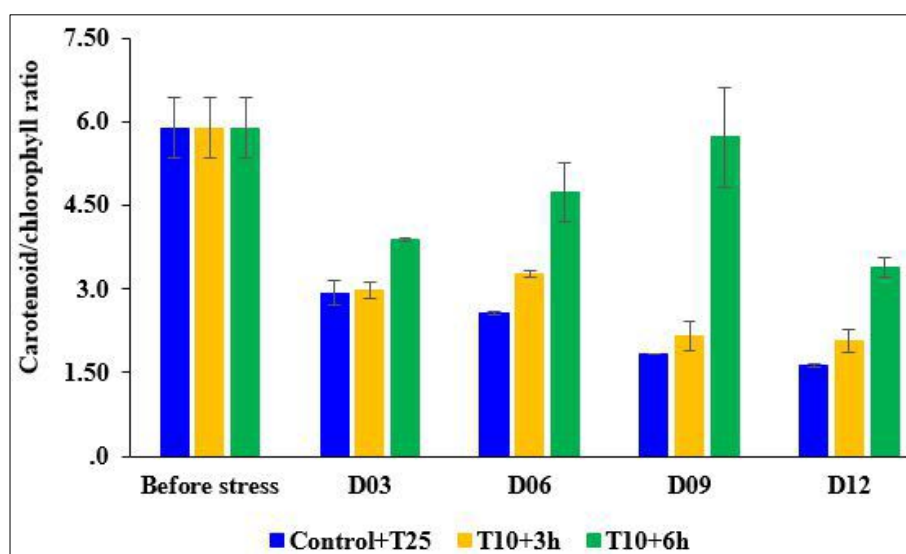


Fig 1.2: Carotenoid/chlorophyll ratio of *D. salina* CCAP 19/18 under different culture conditions temperature inhibition

Total lipid content

Linear regression equation $y=0.0057x-0.0082$ with (y) being the optical density (OD) value and (x) is the total lipid

content ($\mu\text{g/mL}$) with a linear range determined from 10-140 $\mu\text{g/mL}$, correlation coefficient $R^2=0.9992$. The equation meets the linearity requirements (Figure 1.3)

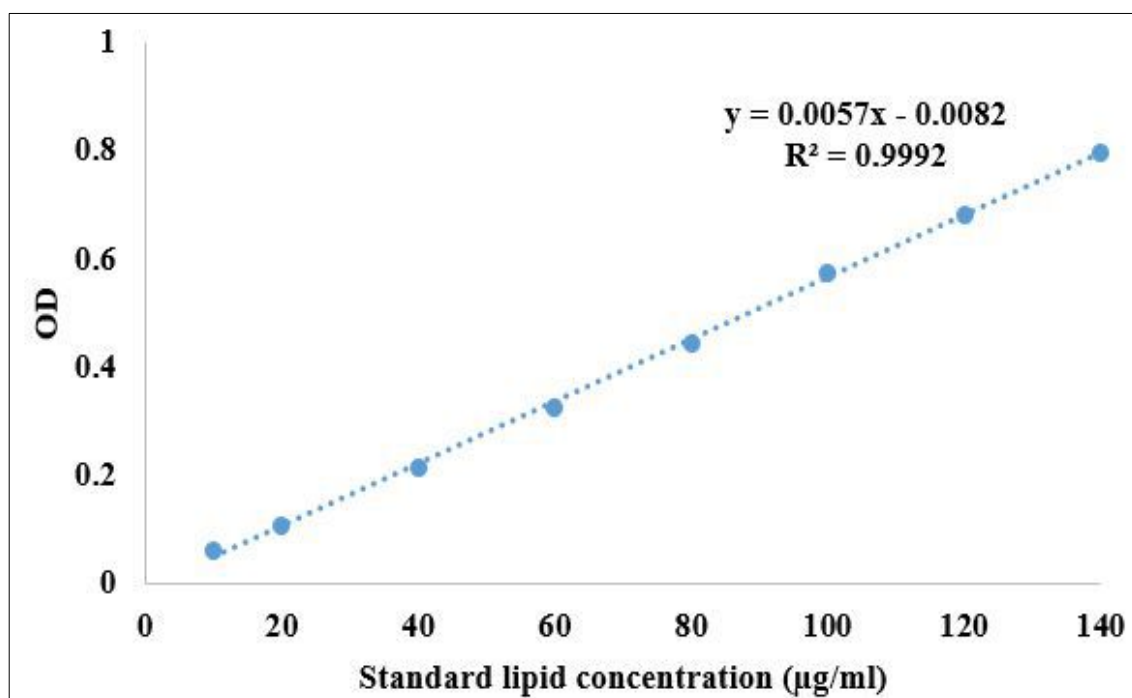


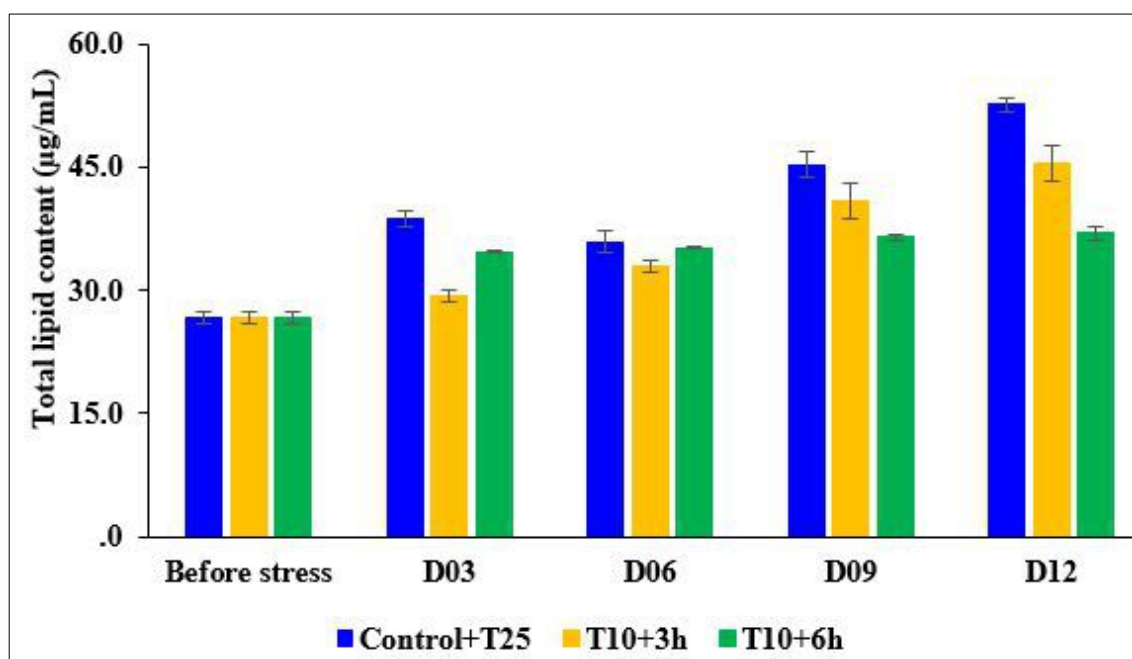
Fig 1.3. Lipid standard curve chart ($\mu\text{g/mL}$)

Figure 1.4 shows that the total lipid content per volume ($\mu\text{g/mL}$) of *D. salina* CCAP 19/18 increased after inhibition and reached the highest value in the control treatment Control+T25 (52.586 $\mu\text{g/mL}$) of day 12 ($p \leq 0.05$), higher than the inhibition treatments T10+3h and T10+6h (Figure 1.4). Meanwhile, the total lipid content (pg/cell) of *D. salina* under the T10+6h inhibition condition reached a higher value than the remaining temperature inhibition culture conditions ($p \leq 0.05$), in which day 12 showed the highest value of 29.209 pg/cell (Figure 1.4). The intermittent exposure to 10°C (6 hours/day) significantly enhanced lipid synthesis and accumulation, resulting increased in cellular

lipid content compared to control conditions ($p \leq 0.05$). Wada and colleagues (1994) researched and discovered that cells produce PUFA to protect the photosynthetic apparatus from the effects of cold temperature stress ^[21].

Total Phenolic Content

Linear regression equation $y=0.0239+0.0283$ with (y) being the optical density (OD) value and (x) is the total lipid content ($\mu\text{g/mL}$) with a linear range determined from 1-50 $\mu\text{g/mL}$, correlation coefficient $R^2=0.9995$. The equation meets the linearity requirements (Figure 1.5)



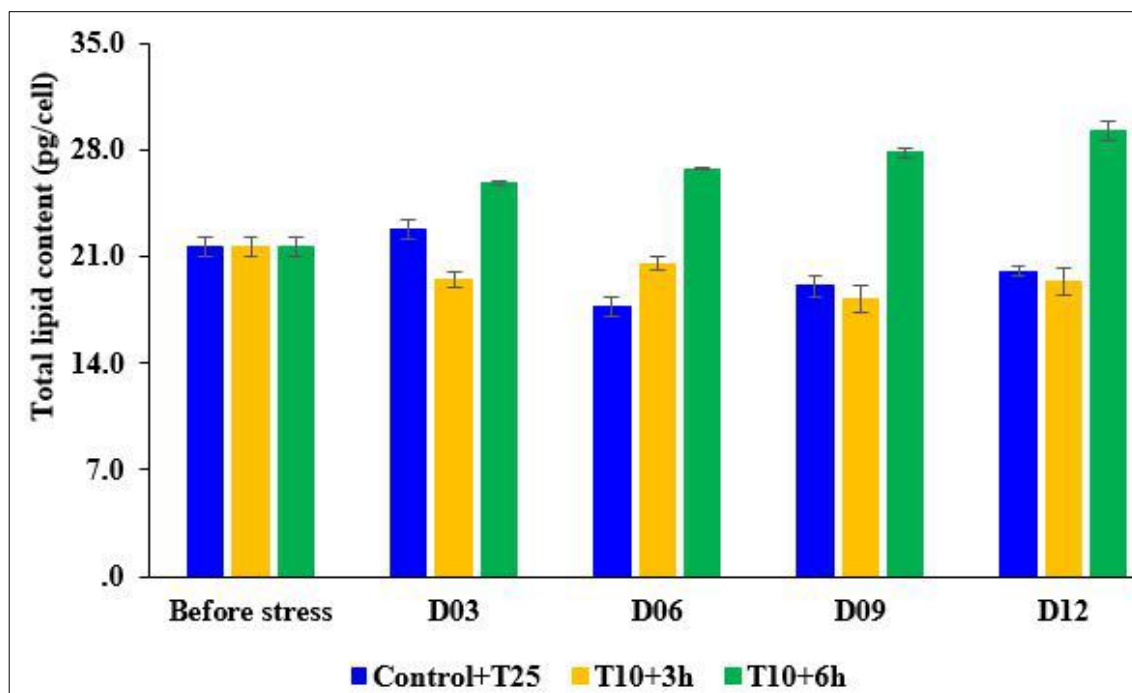


Fig 1.4. Total lipid content ($\mu\text{g/mL}$) and (pg/cell) of *D. salina* CCAP 19/18 under temperature-inhibited culture conditions

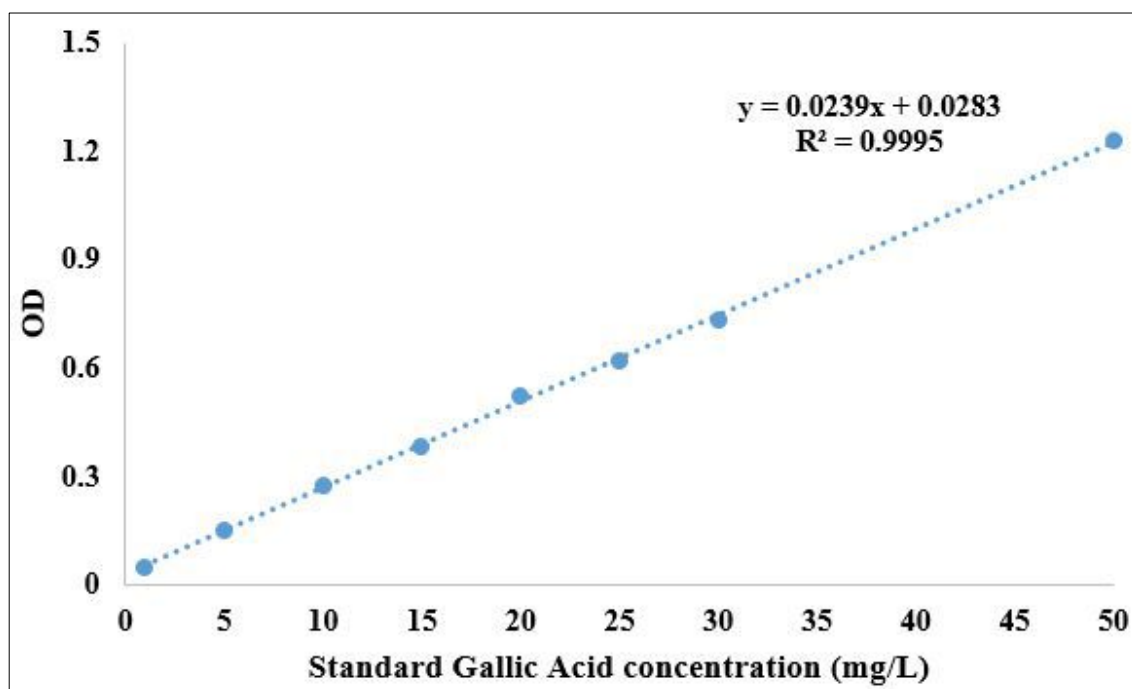


Fig 1.5: Gallic acid standard curve chart (mg/L)

The total phenolic content of *D. salina* CCAP 19/18 increased sharply after temperature inhibition. Specifically, phenolic content ($\mu\text{g/mL}$) increased sharply and reached its maximum on day 12 after inhibition, between the conditions Control+T25, T10+3h and T10+6h with no significant difference ($p=0.057$) (Figure 1.6). The total phenolic content (pg/cell) of CCAP 19/18 increased and reached the highest value in the T10+6h inhibition condition from the

6th day after inhibition and was significantly different from the control and T10+3h conditions ($p \leq 0.05$) (Figure 1.6). Phenolic compounds are one of the substances that play a major role in antioxidant activity in microalgae. Under cold temperature culture conditions, it helps increase the accumulation of phenolic compounds in microalgae to resist stress of cold conditions, content under cold conditions in response to abiotic oxidative stress ^[22].

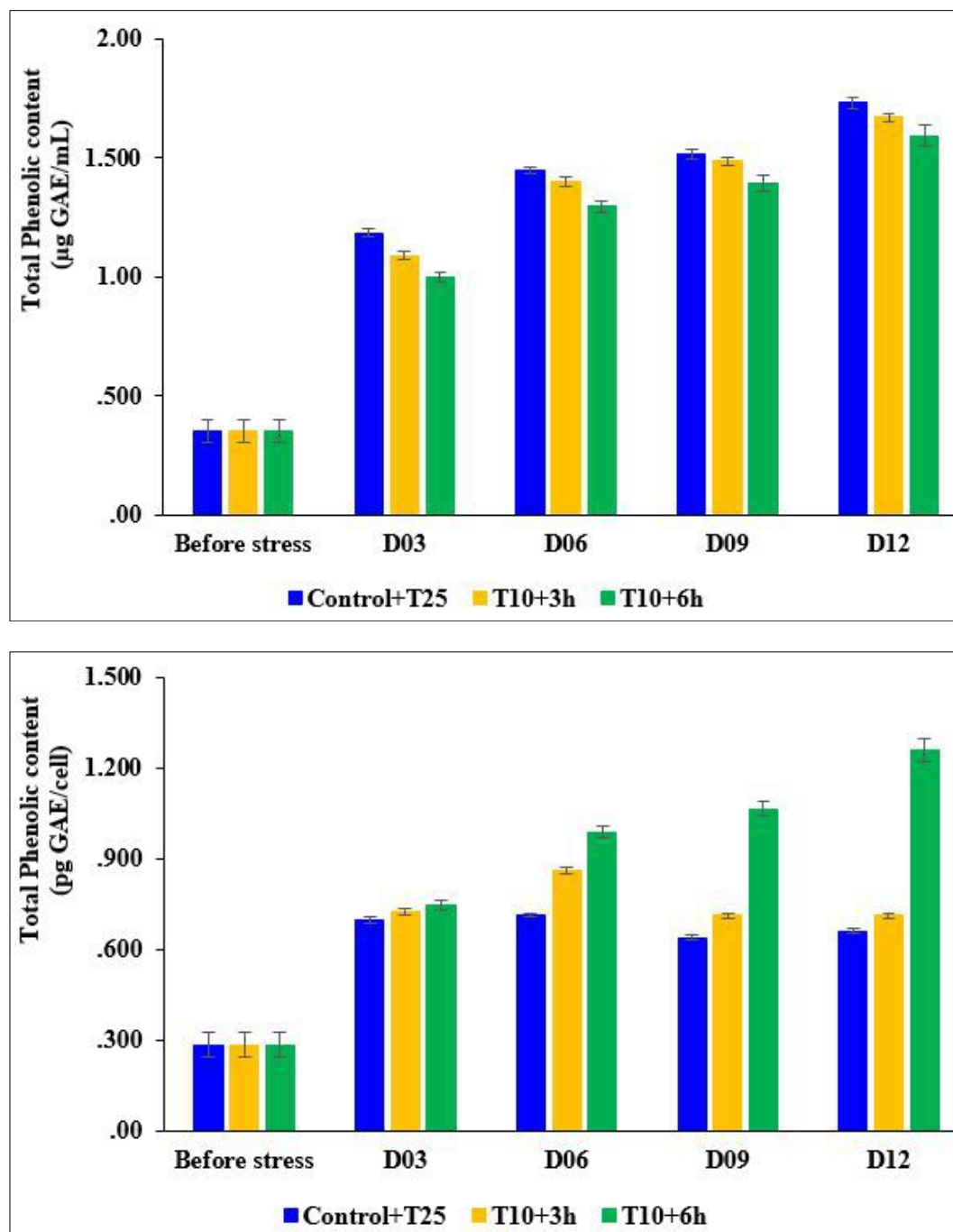


Fig 1.6: Total Phenolic content (µg GAE/mL) and (pg GAE/cell) of *D. salina* CCAP 19/18 under temperature-inhibited culture conditions

Antioxidant Capacity

The antioxidant capacity (I%/mL) of *D. salina* microalgae cultured with temperature inhibition is shown in Figure 1.7. The antioxidant capacity of *D. salina* increased slightly after inhibition and there was no difference significance between culture conditions ($p > 0.05$) (Figure 1.7). The cell antioxidant capacity of *D. salina* CCAP 19/18 reached a high value under the inhibition condition T10+6h (29.255%) on day 12, with a significant difference with the control treatment conditions Control+T25 and T10+3h ($p \leq 0.05$) (Figure 1.7). The relative increase in antioxidant capacity (38.4-40.1% inhibition) was modest compared to the substantial increases observed in carotenoid (290% increase)

and phenolic content (355% increase), suggesting potential temperature dependent variations in the activity of different antioxidant systems. This may be due to the low temperature factor of 10°C affecting the activity of the antioxidant enzyme system of *D. salina*. Free radical neutralization test of *D. salina* CCAP 19/18 shows the antioxidant capacity of substances in microalgae such as carotenoids, phenolic compounds and enzymatic antioxidants produced in chloroplasts and mitochondria under cold stress conditions to fight harmful free radicals of DPPH. Although cold conditions reduce enzyme activity, the antioxidant capacity of carotenoids or phenolic compounds plays their role very well [23].

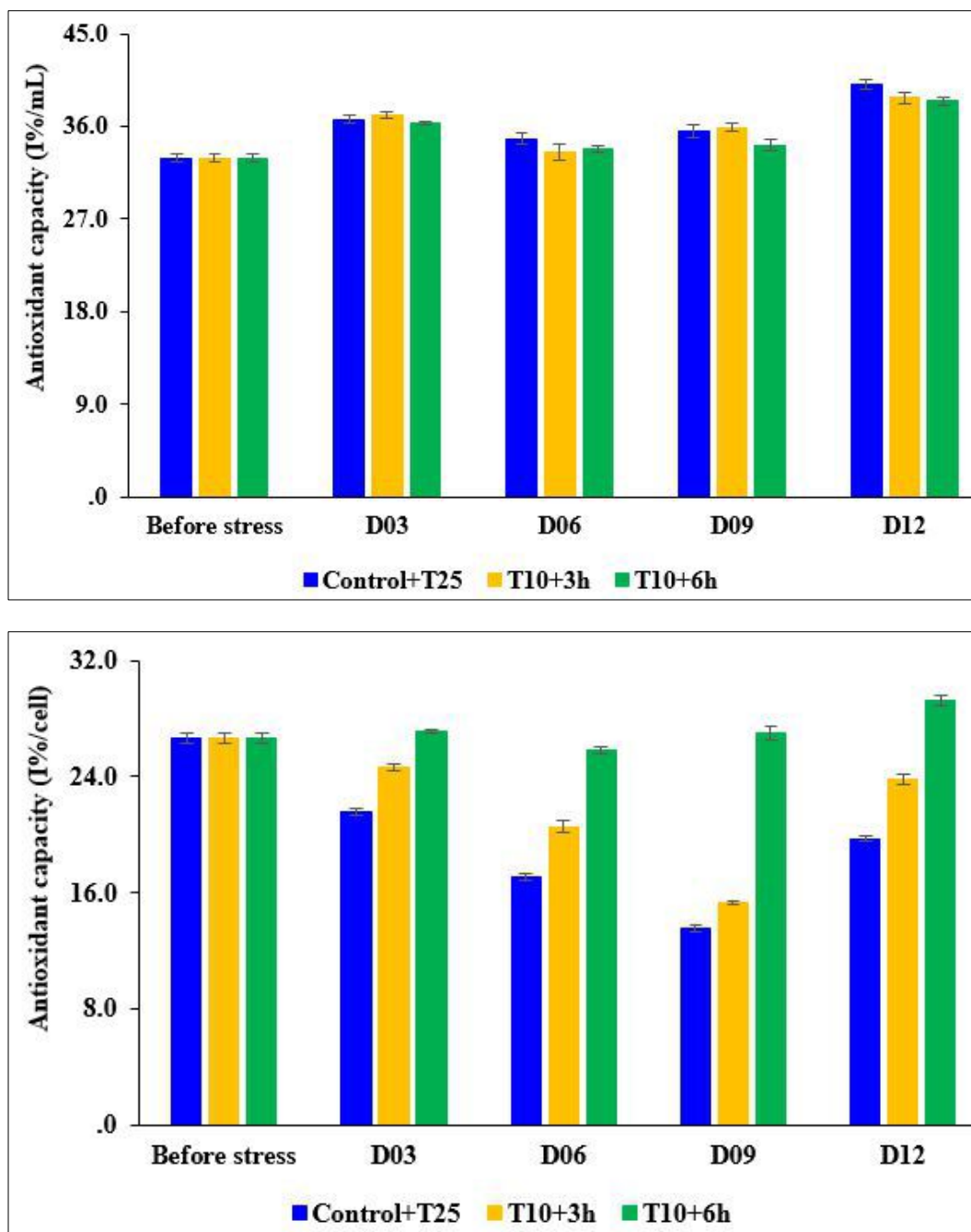


Fig 1.7: Antioxidant capacity (I%/mL) and (I%/cell) of *D. salina* CCAP under temperature-inhibited culture conditions

Conclusion

Microalgae strain *Dunaliella salina* CCAP 19/18 cultured under temperature-inhibited conditions (10°C, 25°C and 45°C) results in the accumulation of high levels of biologically active substances such as carotenoids, phenolic compounds and antioxidant activity in cells. The synthesis and accumulation of carotenoids, lipids, phenolic compounds and antioxidant capacity increased under culture conditions inhibited by cold temperature of 10°C for 6 hours/day in 1,5M RM2 medium. While the inhibitory condition of 45°C causes the cell membrane structure to break down, leading to the complete death of *D. salina* microalgae after 3 days of inhibition.

References

- De Souza Celente G, *et al.* Potential use of microalgae *Dunaliella salina* for bioproducts with industrial relevance. *Biomass Bioenergy*. 2022;167:106647.
- Prieto A, Canavate JP, García-González M. Assessment of carotenoid production by *Dunaliella salina* in different culture systems and operation regimes. *J Biotechnol*. 2011;151(2):180-185.
- Pick U, *et al.* A hypothesis about the origin of carotenoid lipid droplets in the green algae *Dunaliella* and *Haematococcus*. *Planta*. 2019;249:31-47.
- Chaisutyakorn P, Praiboon J, Kaewsuralikhit C. The effect of temperature on growth and lipid and fatty acid composition on marine microalgae used for biodiesel production. *J Appl Phycol*. 2018;30:37-45.
- Wu Z, *et al.* The effects of light, temperature, and nutrition on growth and pigment accumulation of three *Dunaliella salina* strains isolated from saline soil. *Jundishapur J Microbiol*. 2016;9(1).
- Reshma R, *et al.* Enhancement of pigments production in the green microalga *Dunaliella salina* (PSBDU05)

- under optimized culture condition. *Bioresour Technol Rep*. 2021;14:100672.
7. Roy UK, Nielsen BV, Milledge JJ. Antioxidant production in *Dunaliella*. *Appl Sci*. 2021;11(9):3959.
 8. Dolatyari A, Eyvazzadeh O, Nateghi L. Determination of *Dunaliella salina* phenolic compounds in laboratory different conditions; c2022.
 9. Trung VH, Anh NLT, Phúc NTH. Môi trường tiết kiệm cho nuôi cấy vi tảo *Dunaliella salina* quy mô pilot ở Việt Nam. *Tạp Chí Khoa Học*. 2021;18(6):1006.
 10. Andersen RA. *Algal culturing techniques*. Elsevier; c2005.
 11. Shaish A, Ben-Amotz A, Avron M. Biosynthesis of β -carotene in *Dunaliella*. *Methods Enzymol*. 1992;439-444.
 12. Dere Ş, GÜNEŞ T, Sivaci R. Spectrophotometric determination of chlorophyll-A, B and total carotenoid contents of some algae species using different solvents. *Turk J Bot*. 1998;22(1):13-18.
 13. Park J, *et al*. Easy and rapid quantification of lipid contents of marine dinoflagellates using the sulpho-phospho-vanillin method. *Algae*. 2016;31(4):391-401.
 14. Anschau A, *et al*. Validation of the sulfo-phospho-vanillin (SPV) method for the determination of lipid content in oleaginous microorganisms. *Braz J Chem Eng*. 2017;34:19-27.
 15. Michiu D, *et al*. Implementation of an analytical method for spectrophotometric evaluation of total phenolic content in essential oils. *Molecules*. 2022;27(4):1345.
 16. Shah P, Modi H. Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. *Int J Res Appl Sci Eng Technol*. 2015;3(6):636-641.
 17. Paixao N, *et al*. Relationship between antioxidant capacity and total phenolic content of red, rosé and white wines. *Food Chem*. 2007;105(1):204-214.
 18. Orset S, Young AJ. Low-temperature-induced synthesis of α -carotene in the microalga *Dunaliella salina* (Chlorophyta). *J Phycol*. 1999;35(3):520-527.
 19. Potijun S, Yaisamlee C, Sirikhachornkit A. Pigment production under cold stress in the green microalga *Chlamydomonas reinhardtii*. *Agriculture*. 2021;11(6):564.
 20. Ras M, Steyer JP, Bernard O. Temperature effect on microalgae: a crucial factor for outdoor production. *Rev Environ Sci Biotechnol*. 2013;12(2):153-164.
 21. Wada H, Gombos Z, Murata N. Contribution of membrane lipids to the ability of the photosynthetic machinery to tolerate temperature stress. *Proc Natl Acad Sci USA*. 1994;91(10):4273-4277.
 22. León-Vaz A, *et al*. Exploring Nordic microalgae as a potential novel source of antioxidant and bioactive compounds. *New Biotechnol*. 2023;73:1-8.
 23. Huang JJ, Cheung PCK. Cold stress treatment enhances production of metabolites and biodiesel feedstock in *Porphyridium cruentum* via adjustment of cell membrane fluidity. *Sci Total Environ*. 2021;780:146612.