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Effect of Seawater Temperatures on the Growth and Biochemical Profile of the Tropical Green Seaweed, *Caulerpa lentillifera* J. Agardh

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Abstract

Seaweeds are marine algae that are used as a food source, a means of producing hydrocolloids for industrial uses, as well as a source of medication, dietary supplements, fertilizer, and animal feed, particularly in maritime nations. *Caulerpa lentillifera* J. Agardh is an edible green seaweed that has been used as a fresh vegetable and has gained a lot of attention recently due to its nutritional content and beneficial composition for human consumption. *C. lentillifera*, which grows naturally in tropical locations, was first commercially cultivated in the Philippines, then by Japan in the late 1980s, and more recently by Taiwan, China. Numerous studies have revealed that *C. lentillifera* is high in protein, carbohydrates, minerals, vitamins, dietary fibres, polyunsaturated fatty acids, including omega-3 fatty acids, and bioactive compounds such as phenolic compounds, polysaccharides, and siphonaxanthin, which are reported to have high antioxidant, anticancer, anti-diabetic, and immunomodulation properties and potential health benefits. In our current study, the influence of temperature on the growth of *C. lentillifera* was investigated. The experiment was conducted in enriched seawater containing 320μmol/L NO^{3-} and 10.6μmol/L PO_4^{3-} for 30 days at three different temperatures: 20, 24, and 28 ºC. Their growth, photosynthetic pigments, and biochemical compositions, including the contents of protein, carbohydrates, lipids and fatty acids profiling, were determined to investigate the effects of temperatures and nutrients on *C. lentillifera*. In comparison to 24 ºC and 20ºC, our results showed that the highest growth rate and chlorophyll a content were observed at 28 ºC, with 8.77±0.01 % d^{-1} and 3397.02±5.36 μg g^{-1} respectively. *C. lentillifera's* total protein (38.72±1.40% DW^{-1}) and carbohydrate content (61.56±6.73% DW^{-1}) was also found to be highest at 28ºC in comparison to other temperatures. However, the lipid content of *C. lentillifera* was shown to be higher at 20ºC with 8.70 ±0.10 % DW^{-1}. In conclusion, lower temperatures impeded the photosynthetic processes, reduced the amount of soluble protein, and restricted the development rate of *C. lentillifera*, but they also promoted the accumulation of other substances like lipids, fatty acids, and carotenoids. This study is part of an ongoing project to optimize the culture of *C. lentillifera* in the cold, nutrient-rich effluent generated from an ocean thermal energy conversion (OTEC) system.

Key words : Caulerpa lentillifera, Seaweeds, Temperature, Biochemical composition

1. Introduction

Seaweeds are non-flowering photosynthetic marine flora; function as the primary producers in the marine ecosystem. Seaweeds are widely distributed in the intertidal, tidal and subtidal zones of tropical to polar regions. In 2020, the world's annual seaweed production had reached 36,165,326 tonnes FW, of which 96.81% (35,013,088 tonnes

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FW) was produced by the seaweed aquaculture farming industry, and worth an estimated annual value of USD 16,444,926,480. (FAO, 2022a). It is an important marine resource for phycocolloid industries, serving as food for direct human consumption, components for medical, pharmaceutical, nutraceuticals, and cosmetics industries as well as bioresources for aquaculture and agriculture industries (García-Poza et al, 2020). *C. lentillifera* also contains high amount of carbohydrates (phycocolloids such as alginates, agar, carrageenan); proteins, minerals and is also found to be a rich source of important bioactive components.

Tropical seaweed aquaculture farming harvested 99.08 % (15,061,383 tonnes FW) of tropical seaweed production in 2020, worth USD 5,681,861.48. (FAO, 2022b). In 2020, *Caulerpa* aquaculture farming produced a total of 1,021.36 tonnes FW with an annual value of USD 623.34. (FAO, 2022a) *Caulerpa lentillifera* or commonly known as sea grapes, is widely consumed as a fresh vegetable in Asia, specifically in Southeast Asia.

The most abundant contents in *C. lentillifera* are carbohydrates (~43 -55%), and dietary fibre (Insoluble dietary fibres ~ 15.75 - 28.98%). Protein (~ 13-25%), essential minerals (Na, K, Ca, and Mg), vitamins (A and C) are also present (Syakilla et al., 2022). 25 major fatty acids species were identified in *C. lentillifera*, with high omega-3 fatty acids, and polyunsaturated fatty acids, which act as strong antioxidants. Bioactive compounds, such as phenolic compounds, polysaccharides, and siphonaxanthin also found in *C. lentillifera* where these compounds are reported to have high antioxidant, anticancer, anti-diabetic, and immunomodulation properties. Reported health benefits contributed by *C. lentillifera* included cardioprotective properties such as anti-hypertensive and hypolipidemic, antibacterial, anticancer, anti-coagulant, anti-hyperglycemic, anti-diabetic, anti-inflammatory, antioxidative, anti-pyretic, chelating agent, and immunostimulatory (- innate immune system) (Syakilla et al., 2022)

Ocean thermal energy conversion (OTEC) is an emerging technology for renewable energy production. The DSW that is drawn up, generate energy through the difference in temperature between the cold DSW and the warm surface water. The cold deep seawater applications from the OTEC effluents can be used in different applications and industries including seaweed cultivation. It is very useful for extending the range of marine organisms that can be cultivated and temperate species of algae can now be cultivated in the tropics, using these effluents. The cultivation of seaweeds in the effluents arising from an OTEC system and the valorisation of the seaweed biomass in the form of high-value products, would contribute to the sustainability of the energy production system. Furthermore, integrating the OTEC and seaweed cultivation may pave the way for the development of new algal products and the expansion of the algal industry.

Integration of *C. lentillifera* cultivation in OTEC industries may provide more avenues for research and product development, as *C. lentillifera* can be considered a promising and valuable alternative product with high nutritional value for the future. For example, a lower temperature of OTEC effluent may increase lipid and fatty acid production because desaturation of saturated fatty acids is reduced and production of unsaturated fatty acids, consisting of MUFAs and PUFAs, is increased, which is important in the medicine, cosmetics, and biotechnology industries. Hence, culturing *C. lentillifera* in cold OTEC effluent will greatly contribute to the production of valuable products and eventually improve its market value.

2. Research Objective and Scope of studies

The objectives of this project are to investigate the effect of seawater temperature and nutrients (Nitrate (NO_3^-) & phosphate (PO_4^{3-}) on the growth and biochemical profiles of selected commercial seaweed, *C. lentillifera*. The purpose of these studies is to provide a baseline for the land-based cultivation and utilization of cold deep seawater sources such as effluent from the OTEC system. This can serve as a sustainable method to produce more biomass and higher desired quality products from the seaweed. Besides, the environmental factors such as light, nutrients, temperature and salinity can be controlled and manipulated throughout the cultivation period compared to an open cultivation system like an offshore/ near-shore cultivation system. These can help to improve and holds the potential of a year-round production especially for targeted specific valuable molecules. In addition, a cleaner and marketable biomass

3. Research methodology

3.1 Seaweed collection & maintenance

Caulerpa lentillifera were collected from natural seaweed beds in Port Dickson (02˚26'38" N; 101˚51'21" E). The collected seaweeds were transported in a healthy state in a cool Styrofoam box. The stocks were rinsed with seawater while epiphytes, dirt and plants parts with any signs of de-pigmentation and necrosis were removed before being used in the experiments. Healthy cleansed seaweeds were cultured in tanks of aerated seawater (30ppt) and maintained at 28ºC, 25µmol m^{-2} s^{-1} with 12 h light and 12 h dark cycles.

3.2 Experimental Design

Growth studies of *Caulerpa lentillifera* were conducted in a 30 L tank in a growth incubator with 50 µmol m^{-2} s^{-1} illuminated with cool-white LED tubes and 12h L: 12h D cycles for 30 days. The experiments were carried out in five replicates in 20 litters of 30 ppt artificial seawater (Instant Ocean® Synthetic Sea Salt) enriched with 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^-, with a starting inoculum of 100 g per replicate for each of the three temperature treatments (20, 24, and 28 ºC). Samples were harvested from each tank on day 0, 6, 12, 18, 24 & 30 for growth & biomass determination, and biochemical (carbohydrate, protein, lipid and pigment) extraction and determination.

3.3 Biomass and relative growth rate (RGR):

The fresh weight (g) and relative growth rate (%d^{-1}) of *C. lentillifera* were measured at six-day intervals for 30 days. The relative growth rate (RGR) regarding day 0 (RGRi) and previous samples (RGR_{t-1}) were calculated using the following equations (Phang, 1997):

$$RGR_i\,(\%\ d^{-1}) = [(W_t - W_i)/\ (W_i * \Delta t)] * 100 \quad (1)$$

Where W_i is the initial fresh weight, W_t is the fresh weight on day t, Δt is the time interval

$$RGR_{t-1}\,(\%\ d^{-1}) = [(W_t - W_{t-1})\ (W_{t-1} * \Delta t)] * 100, \quad (2)$$

Wt is the fresh weight on day t and W_{t-1} is the fresh weight on the previous sampling day, Δt is the time interval

3.4 Extraction of chlorophyll-a & carotenoid content:

The determination of chlorophyll-a content and carotenoid was done using the colorimetric method by (Strickland and Parson, 1972). The supernatant was measured at OD_{665nm}, OD_{645nm}, and OD_{630nm} for chlorophyll –a content and OD_{452nm} for carotenoid content.

3.5 Biochemical composition extraction of *Caulerpa lentillifera:*

For the biochemical composition assay, the *C. lentillifera* extracted based on the standard method such as Protein (Bradford, 1976), carbohydrates (Dubois et al., 1956), lipid (Bligh and Dyer 1959) and fatty acid transesterification (Christie et al., 1989) were determined on the day 0,12,18, 24 and 30 of the experiments. The protein, carbohydrates and lipid content of *C. lentillifera* were analyzed and expressed in percentages dry weight (% DW^{-1}). The analysis of fatty acids was conducted using gas chromatography for the determination of fatty acid composition (Christie et al., 1989).

3.6 Statistical Analyses

Tukey's – HSD comparisons were applied to determine statistically significant differences ($p<0.05$) among time and treatments following ANOVA. One-way ANOVA was used for growth and biochemical analyses of C. *lentillifera* at the end of the experiments. A significance level of 95% ($p<0.05$) was set for all the tests. Data are presented as mean ± SD (the number (n) of replicates is presented in each figure caption). The statistical analyses were carried out using

Statistica software version 8.0

4. Results and Discussion

4.1 The growth and biomass production

The highest biomass production of *C. lentillifera* was obtained at 28 ºC with 199.39±3.90 g FW on day 24 compared with 20 ºC & 24 ºC. However, the highest biomass productivity of *C. lentillifera* was also obtained at 28 ºC but on day 6 of 141.48±0.23 g m^{-2} d^{-1} with the relative growth rate of 8.77±0.01% d^{-1}. On contrary, to the biomass production under lower temperature of 20 ºC has it is dropped from 100.66±0.18 g FW on day 0 to 63.68±4.41 g FW on day 6. The low growth rate at 20 ºC could be a result of inadequate acclimatization, which has led to the culture becoming stagnant. Besides, the temperature shock due to inadequate acclimatization also led to the disintegration of the algal thallus and later reduced their biomass production. Hence, it is shown that the growth rate and biomass production of *C. lentillifera* cultivated at the lower temperatures of 20 ºC and 24 ºC exerted a stronger influence to the seaweed production.

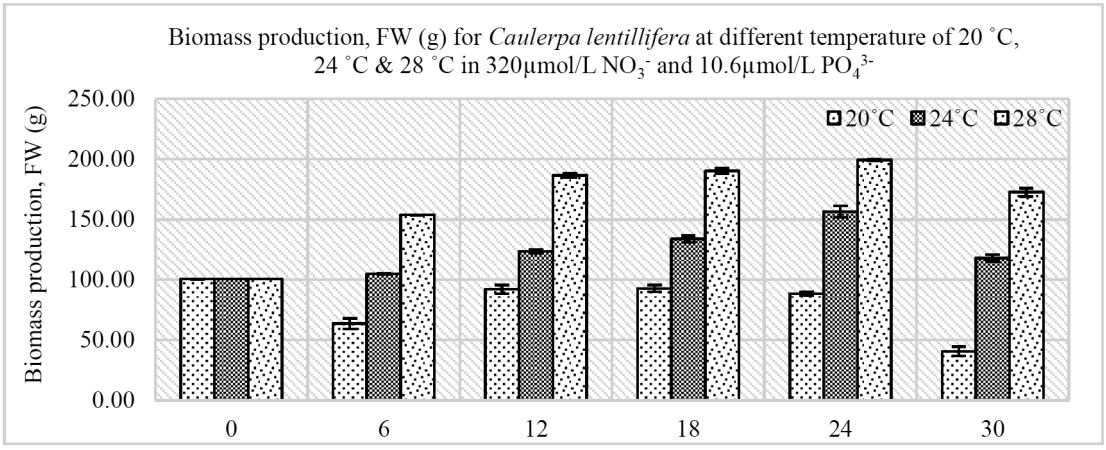

Fig. 1 Biomass production. FW (g) for *Caulerpa lentillifera* at different temperature of 20 ºC, 24 ºC and 28 ºC in 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^{3-}. Data are means ± SD of five replicates (n = 5). The significantly difference was labelled with an asterisk (*), ($p<0.05$).

Table 1 Biomass productivity (g m^{-2} d^{-1}) and relative growth rate (% d^{-1}) for *Caulerpa lentillifera* at different temperature of 24 ºC and 28 ºC in 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^{3-}. Data are means ± SD of five replicates (n = 5). The significantly difference was labelled with an asterisk (*), ($p<0.05$).

	Temperature	
	24 ºC	28 ºC
Biomass productivity (g m^{-2} d^{-1}) Day 6	10.33±0.24	141.38±0.23*
Biomass productivity (g m^{-2} d^{-1}) Day 18	29.30±2.55	79.60±1.86
Biomass productivity (g m^{-2} d^{-1}) Day 24	37.13±3.17	65.73±0.52
Relative growth rate (% d^{-1}) Day 6	0.64±0.01	8.77±0.01
Relative growth rate (% d^{-1}) Day 18	2.16±0.57	4.94±0.11
Relative growth rate (% d^{-1}) Day 24	2.56±0.26	4.04±0.10

4.2 Pigments production

The higher chlorophyll-a content was observed at 28 ºC with 3397.02±5.36 µg g^{-1} and 24 ºC with 3137.27±109.69 µg g^{-1}. On the contrary, at the lower temperature of 20 ºC, the chlorophyll-a content decreased under longer exposure to the lower temperature, as lower temperatures might suppress the enzyme activity in chlorophyll synthesis (Guo et al., 2015) and disrupt the algal cell component for photosynthesis (Allen et al., 2001). Lowering the temperature generally

reduces the reaction rates and therefore limits the sinks for the absorbed excitation energy (light), particularly in CO_2 fixation and photorespiration. Smaller sinks for absorbed excitation energy increase the potential for oxidative damage to PSII (Allen et al., 2001) hence affects chlorophyll production and photosynthesis. As *C. lentillifera* grown at 20ºC became feebler, the branches and the fronds had lost their pigmentation, became perforated and fragile. which later led to the disintegration of the algal cells (Andersen et al., 2013).

However, the highest carotenoid content was found at 20 ºC with 2399.40±36.40 µg g^{-1}. Carotenoid is an auxiliary pigment that aids in the maintenance the photosynthetic stability, especially in adverse situations. Hence, more accumulation of carotenoid was observed at 20 ºC than its ambient temperature to protect its photosynthetic mechanism.

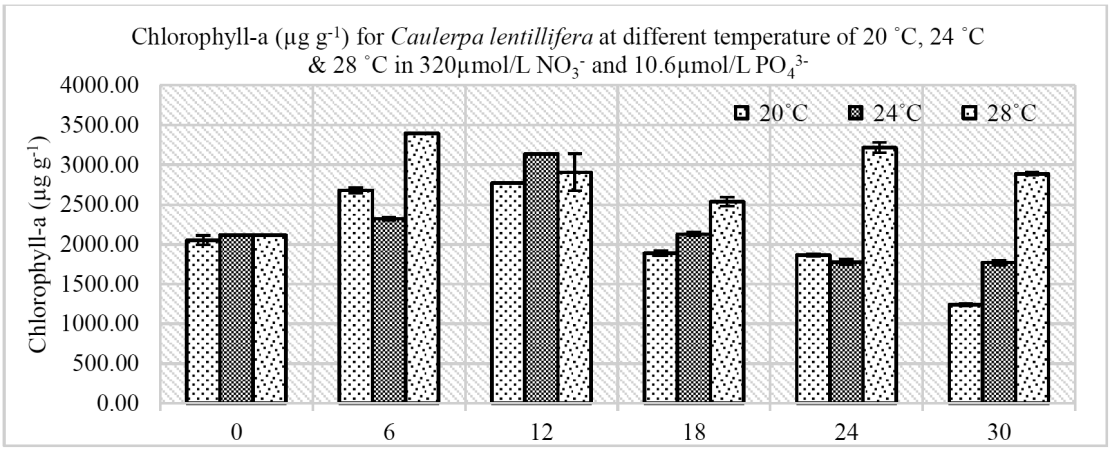

Fig. 2 Chlorophyll-a content (µg g^{-1}) for *Caulerpa lentillifera* at different temperature of 20ºC, 24ºC and 28ºC in 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^{3-}. Data are means ± SD of five replicates (n = 5). The significantly difference was labelled with an asterisk (*), ($p<0.05$).

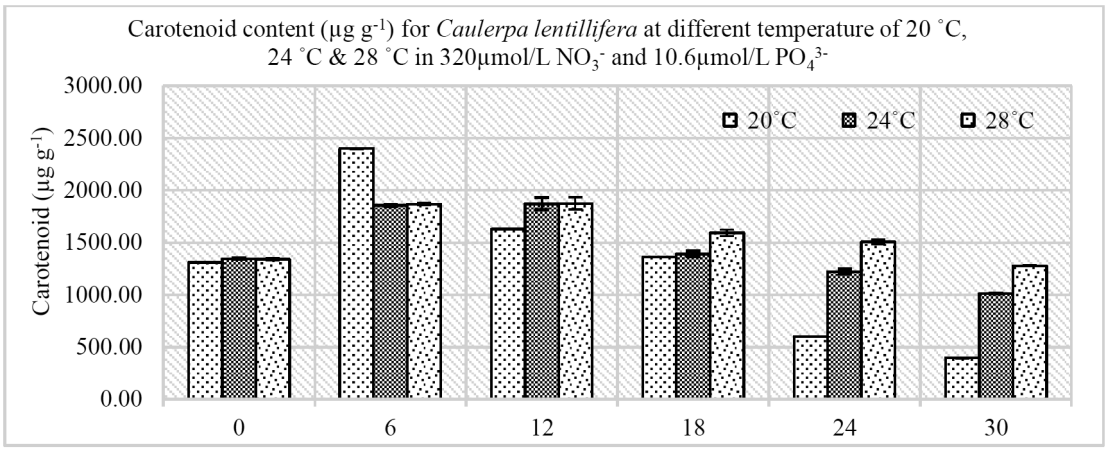

Fig. 3 Carotenoid content (µg g^{-1}) for *Caulerpa lentillifera* at different temperature of 20 ºC, 24 ºC and 28 ºC in 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^{3-}. Data are means ± SD of five replicates (n = 5). The significantly difference was labelled with an asterisk (*), ($p<0.05$).

4.3 Biochemical composition (carbohydrates, protein and lipid) production

The highest total carbohydrate content was obtained in *Caulerpa lentillifera* at 28 ºC with 61.56±6.73% DW^{-1} followed by 24 ºC with 59.24±6.92% DW^{-1} both on day 18. The carbohydrate accumulation of compatible solutes occurred at a lower temperature to maintain their cell osmolality and to regulate their photosynthetic mechanism. This is important to maintain the seaweed's energy production and consumption in the algae cells. (Klähn and Hagemann 2011; Ras et al., 2013; Barati et al., 2019)

As for protein content, the higher accumulation was also observed at 28 ºC with 38.72±1.40% DW^{-1} and at 24 ºC with 35.35±0.27% DW^{-1} respectively. Studies conducted by Settamongkol et al., 2015, also showed similar protein

accumulation compared to this study, where the protein content found in the green algae species such as *C. taxifolia* and *C. linum* had 33.83±0.21% DW^{-1} and 30.70±0.70% DW^{-1} respectively. This showed that temperature changes will affect the stability of cellular components, protein's membrane and lessen the protein synthesis through protein

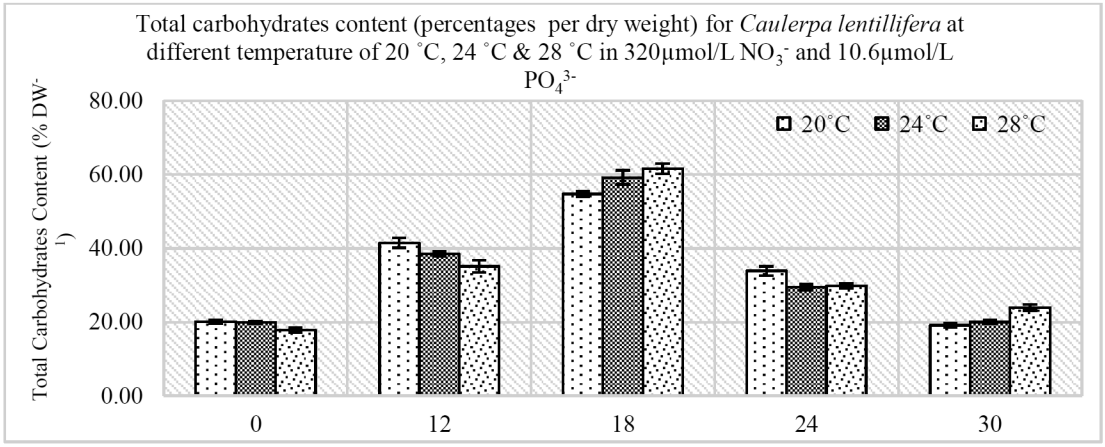

Fig. 5 Total carbohydrates content (% DW^{-1}) for Caulerpa lentillifera at different temperature of 20 ºC, 24 ºC and 28 ºC in 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^{3-}. Data are means ± SD of five replicates (n = 5). The significantly difference was labelled with an asterisk (*), ($p<0.05$).

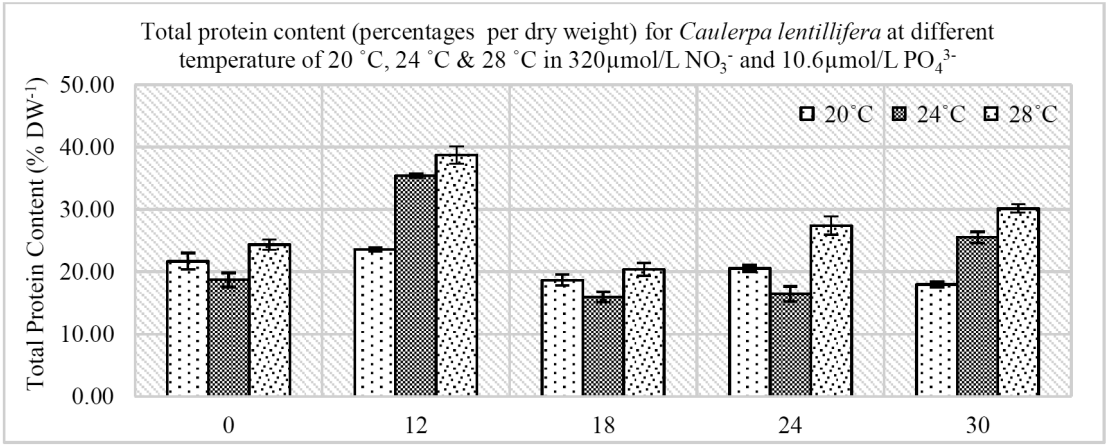

Fig. 6 Total protein content (% DW^{-1}) for Caulerpa lentillifera at different temperature of 20 ºC, 24 ºC and 28 ºC in 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^{3-}. Data are means ± SD of five replicates (n = 5). The significantly difference was labelled with an asterisk (*), ($p<0.05$).

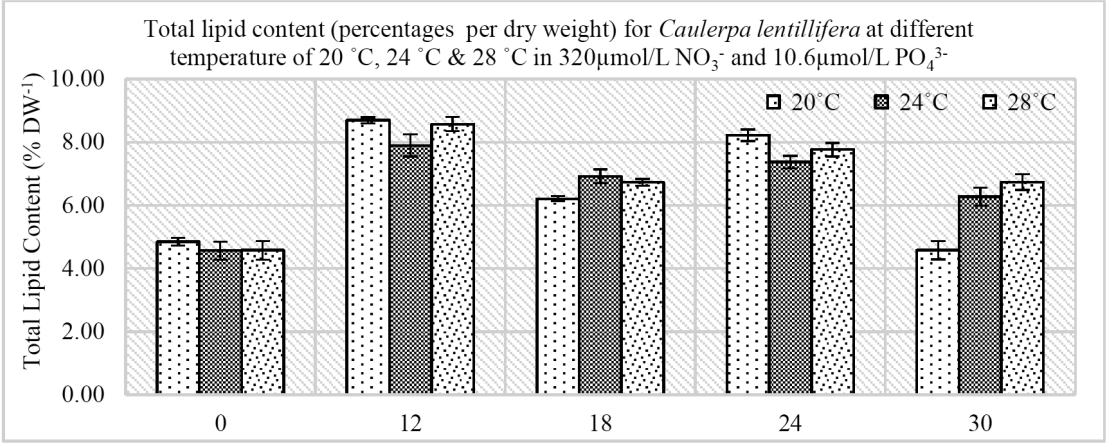

Fig. 7 Total lipid content (% DW^{-1}) for Caulerpa lentillifera at different temperature of 20 ºC, 24 ºC and 28 ºC in 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^{3-}. Data are means ± SD of five replicates (n = 5). The significantly difference was labelled with an asterisk (*), ($p<0.05$).

modulation and modify their environment in the algal cells (Eggert 2012; Wagenen et al., 2012; Schroda et al., 2015; Barati et al., 2019).

The lipid content of *C. lentillifera* obtained at 20 ºC ranged from 4.84 ±0.13 % DW^{-1} to 8.70 ±0.10 % DW^{-1}, followed by *C. lentillifera* cultivated at 28ºC with a similar range of 4.58 ±0.29 % DW^{-1} to 8. 57 ±0.23 % DW^{-1}. The higher lipid accumulation at the lower temperature of 20 ºC showed that, temperature has significant effects on the synthesis and accumulation of lipids and fatty acids. This is because, under stress management and repair strategies, seaweed modifies its lipid biosynthesis pathways to produce and promote accumulations of neutral lipids in the form of triacylglycerol. It offers a carbon and energy storage function in the cell that allows algal to tolerate unfavorable environmental conditions (Hu et al., 2008; Chen et al., 2011; Feng et al., 2011; Mairet et al., 2011; Fakry et al., 2015).

4.4 Fatty Acid distribution

About 25 major fatty acid species were identified in *C. lentillifera* cultured under different temperature treatments of 20 ºC, 24 ºC, and 28 ºC. Among these, C16:0 was the most abundant fatty acid (29.62±0.42% to 40.50±0.16%), followed by C18:0 (9.09±0.34% to 17.61±1.16%), and C20:1n9 (3.95±0.37% to 14.39±0.19%) for all the treatments.

The extent of unsaturated fatty acids increased for cultivated *C. lentillifera* at 20 ºC and in other words, the amounts of polyunsaturated fatty acids (PUFA) were highest in *C. lentillifera* at 20 ºC (18.40±0.35%), followed by 24 ºC (13.07±0.61%), and 20 ºC (12.43±0.51%). In particular, C20:1n9-EPA contributed the highest value in total PUFA species (up to 14.39±0.19%) in *C. lentillifera* cultured at 20 ºC.

The results indicated that, when *C. lentillifera* was exposed to a lower temperature, the PUFA amount increased rapidly to maintain the cell membrane fluidity. This is because, the desaturation of existing C18:0 fatty acid chains or

Table 2 Total fatty acid distribution percentages (%) for *Caulerpa lentillifera* at different temperature of 20 ºC, 24 ºC and 28 ºC in 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^{3-}. Data are means ± SD of five replicates (n = 5). The significantly difference was labelled with an asterisk (*), ($p<0.05$).

	Temperature		
% Fatty acid distribution	**20 ºC**	**24 ºC**	**28 ºC**
SFA	60.85±0.80	68.12±0.69*	68.77±0.34*
MUFA	20.75±0.72	18.81±0.91	18.80±0.46
PUFA	18.40±0.35*	13.07±0.61	12.43±0.51

Table 3 The major species distributed in fatty acid distributions (%) for *Caulerpa lentillifera* at different temperature of 20 ºC, 24 ºC and 28 ºC in 320 µmol L^{-1} NO_3^- and 10.6 µmol L^{-1} PO_4^{3-}. Data are means ± SD of five replicates (n = 5). The significantly difference was labelled with an asterisk (*), ($p<0.05$).

		SFA		**MUFA**		**PUFA**
Temperature	**Day**	**C16:0**	**C18:0**	**C16:1**	**C18:2n6c**	**C20:1n9**
	0	31.22±0.66	11.30±0.11	4.85±0.21	6.04±0.82	10.10±0.42
20 ˚C		34.04±1.93	10.99±0.90	5.25±0.12	6.50±0.57	10.64±0.26
24 ˚C	12	38.59±0.73	10.93±0.16	5.53±0.23	5.10±0.08	10.22±0.22
28 ˚C		40.50±0.16*	9.09±0.34	4.46±0.24	5.85±0.26	8.42±0.29
20 ˚C		34.28±0.23	9.81±0.26	5.75±0.28	4.94±0.30	14.39±0.19*
24 ˚C	18	39.25±0.31	11.37±0.21	5.20±0.26	4.74±0.55	9.22±0.18
28 ˚C		36.35±1.01	9.21±1.03	5.42±0.26	5.51±0.32	7.47±0.46
20 ˚C		32.81±0.67	11.24±0.52	6.59±0.36	4.84±0.15	5.13±0.45
24 ˚C	24	31.55±1.94	11.69±0.75	3.54±1.02	3.88±0.39	4.62±0.13
28 ˚C		36.87±0.50	14.56±1.20	5.60±0.53	3.81±0.06	5.03±0.40
20 ˚C		29.65±1.77	15.06±0.55	3.44±0.30	3.65±0.10	5.50±0.54
24 ˚C	30	29.62±0.42	17.61±1.16*	4.52±0.33	2.08±0.44	4.33±0.35
28 ˚C		33.58±0.64	17.11±1.45	3.63±0.34	2.46±0.24	3.95±0.37

of *de novo* synthesized fatty acids increases as the enzymes encoding the desaturation of this fatty acids such as Δ9 and Δ12 desaturases, are elevated under cold stress conditions (Cao et al., 2017). Hence, temperatures showed a positive relationship with a higher degree of fatty acid desaturation in both cold and freezing environments (Zhang et al., 2012; Barati et al., 2019). Fatty acid distribution varied with the changes in the natural environment, like temperature stress, to maintain their membrane integrity and act as one of the central mechanisms for seaweeds. The mechanism included fatty acid composition, unsaturation of the cells, and re-distribution of fatty acid species in membrane lipids (Cao et al., 2017).

5. Conclusion

Caulerpa lentillifera can be considered a promising and valuable alternative product for the future with high nutritional value. A marine producer with a unique structure and biochemical composition could be exploited for its various properties, such as food, energy, medicine, cosmetics, and biotechnology. Lower temperatures may induce changes in seaweed growth and their biochemical composition, especially for tropical *C. lentillifera*. Under low temperatures, saturated fatty acids reduced and increased the production of unsaturated fatty acids, consist of MUFAs and PUFAs and other seaweed compositions such as lipid and carotenoid productions. This will greatly contribute to the production of valuable lipids and fatty acids in *C. lentilifera*, improving its market value. As a result, cultivating *C. lentillifera* at low temperatures may open up new avenues for research as well as product development in a variety of fields for new sources and the production of high-value products. For example, implementation in OTEC industries application.

6. Acknowledgement

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