

Full Length Research Paper

# Fatty acids composition of microalgae *Chlorella vulgaris* can be modulated by varying carbon dioxide concentration in outdoor culture

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Varying culture methods of *Chlorella vulgaris* (CV) has been associated with different nutrient composition. The aim of this study was to investigate the fatty acid contents and other nutrients of CV subjected to various culturing conditions. We found that CV cultured under 24 h light and 10% CO<sub>2</sub> showed the best growth rates and contained higher lipid, protein and moisture contents compared to other culture conditions. Interestingly, the content of fatty acids of CV was dependent on the amount of CO<sub>2</sub>. Fatty acid analysis of CV by gas chromatography-mass spectrometry (GC-MS) showed the presence of cis-10-pentadecanoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), linolenic acids (C18:3n3) and arachidic acid (C20:0). Remarkably, polyunsaturated fatty acids (linoleic and linolenic acids) are found in abundance compared to other fatty acids in CV. The concentrations of palmitic, oleic, linoleic and linolenic acids increased when the amount of carbon dioxide was raised from 1 to 10% under both culture conditions (12 and 24 h light). This study shows the possibility of modifying lipid contents in freshwater microalgae by varying the amount of carbon dioxide and light.

**Key words:** *Chlorella vulgaris*, microalgae, fatty acids, vitamins, minerals, carbon dioxide.

## INTRODUCTION

Microalgae are capable of producing valuable metabolites, such as proteins, carbohydrates, lipids and vitamins for feed additive, pharmaceutical and nutraceutical purposes (Guerin et al., 2003; Hu, 2004). They have diversified use ranging from supply of fatty acids and vitamins for fish in aquaculture systems (Yoshimatsu et al., 1997; Hamasaki et al., 1998; Gapasin et al., 1998),

daily nutritional supplements, components in foodstuffs, and in the cosmetics industry. *Chlorella* is a genus of single-celled green algae measuring about 2 to 10 µm in diameter and can be found in both fresh and marine water (Kay, 1991). It depends on photosynthesis for growth and multiplies rapidly, requiring only carbon dioxide, water, sunlight and a small amount of minerals. *Chlorella vulgaris* first isolated by Beijerinck in 1890 is a rich source of proteins, vitamins (B-complex and ascorbic acid), minerals (potassium, sodium, magnesium, iron and calcium), β-carotene, chlorophyll, *Chlorella* growth factor (CGF) and other beneficial substances (Konishi et al., 1996). The nutritional value of microalgae is influenced

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by their size, shape, digestibility and biochemical composition (Webb and Chu, 1983; Brown et al., 1989). The components in algae such as *Spirulina* which are responsible for the therapeutic values are PUFA, phycocyanin (Estrada et al., 2001) and phenolics (Miranda et al., 1998). In recent years, fatty acids compositions in large scale production of microalgae including marine algae have created considerable interest among researchers.

This is mainly because of the health benefit of mono and polyunsaturated fatty acids (MUFA and PUFA) that can be found in plants including microalgae. Consumption of n-3 PUFAs from both seafood and plant sources may reduce coronary heart disease (CHD) risk as reported by Mozaffarian et al. (2005) in a cohort study of 45,722 men. Thus, many health supplement stores now sell preparation of microalgae such as spirulina and chlorella packed in capsule or caplets, or even in food and beverages known to have therapeutic values in treating hypercholesterolemia, hyperlipidaemia and atherosclerosis (Ramamoorthy and Premakumari, 1996; Eussen, 2010). The fatty acid contents of microalgae are influenced by the environmental and cultural condition selected for its growth (Petkov and Garcia, 2007). Some of the environmental conditions include heterotrophic, photoautotrophic and nitrogen deprivation or stimulation (Vladimirova et al., 2000; Piorreck et al., 1984; Dickson et al., 1969; Nichols and Bold, 1965). Although protein and carbohydrate compositions have been studied for CV, however, little information is available on the effect of light and carbon dioxide in modulating the fatty acid composition of this microalga. Carbon dioxide-enriched air has long been employed as a carbon source in the growth of unicellular, photosynthesizing algae, especially for physiological experimentation only (Dickson et al., 1969). In this study, we investigated the composition of lipid and fatty acids of CV cultured in low and high CO<sub>2</sub> concentrations.

## MATERIALS AND METHODS

### Microorganism and growth conditions

*C. vulgaris* Beijerinck 072 was obtained from the University of Malaya Algae Culture Collection (UMACC) and grown outdoor in a large tank with Bold's basal medium (BBM) of Nichols and Bold (1965). The pH was adjusted to 6.8 while the temperature was maintained at 25°C. The culture was subjected to two different culture conditions, in terms of the duration of illumination and the percentage of CO<sub>2</sub> supplied into the media. This was done for the purpose of optimizing the culture conditions. CV was cultured under 12 h natural sunlight with 1% CO<sub>2</sub> and 10% CO<sub>2</sub>, and 24 h of light (12 h natural sunlight during the day and 12 h 40 W fluorescent light at night). The CV tank was designed by the researchers in this study and it was made of Plexiglas, measuring 1.3 m in radius and 0.3 m in depth, which amounts to 1000 L in volume. The media was constantly mixed by a paddle-wheel to prevent sedimentation of the algae and to ensure that all algal cells were equally exposed to the light and nutrient. The tank was covered with Plexiglas to avoid contamination from the environment and simultaneously to utilize

sunlight during the day. The biomass of CV was routinely monitored for growth by measuring optical density via spectrophotometer (SHIMADZU, JAPAN) at 680 nm to ensure an exponential phase of growth and was maintained until it reached the stationary phase. Microbial contamination was monitored by microscopical examination. CV was harvested just before the stationary phase by sedimentation process and centrifugation at 1000 rpm for 15 min to separate the algae and the media. The biomass cake was washed with distilled water to remove non-biological material such as salt precipitates. CV slurry was then freeze-dried for storage.

### Moisture analysis

The moisture content of CV was determined by drying a representative 1 g sample in an oven with air circulation at 100 ± 3°C overnight (AOAC, 1995).

### Determination of ash

The ash contents were estimated by heating the CV overnight in a furnace at 550°C (AOAC, 1995).

### Crude protein analysis

This was estimated by the Kjeldahl method using a 2200 KJELTEC system distilling unit (TECATOR, HOGANAS, SWEDEN). The crude protein content was calculated by multiplying the nitrogen content by a factor of 6.25 (AOAC, 1995).

### Extraction of crude lipids

Crude lipids were extracted from the CV powder in a soxhlet extractor (2055 SOXTEC AVANTI SYSTEM; TECATOR, HOGANAS and SWEDEN). The contents of crude lipids were determined gravimetrically after oven-drying (100°C) the extract for 15 min.

### Total dietary fiber analysis

The content of total dietary fiber (TDF) in CV was determined according to the AOAC enzymatic-gravimetric method (AOAC, 1995). In brief, four replicates of samples (1 g of dry matter) were first treated with two amylases; a heat-stable  $\alpha$ -amylase (catalog no. A3306, SIGMA, USA) for 15 min in a boiling water bath and a fungal amyloglucosidase (from *Aspergillus niger*, catalog no. A9913, SIGMA, USA) for 30 min at 60°C to remove starch and then a bacterial protease (from *Bacillus licheniformis*, catalog no. P3910, SIGMA, USA) to lyse the protein. The enzyme-treated mixture containing the buffer solution and non-digestible materials was precipitated with 95% ethanol. Then, the ethanol-insoluble residue was filtered with a Fibertec System (TECATOR 1023, SWEDEN). The residue recovered was washed, oven-dried and weighed to give the gravimetric yield of the seaweed fiber material or TDF. The weight of TDF was corrected for ash and residual protein content and a blank.

### Total carbohydrate

The total carbohydrate content in CV was calculated by the subtraction technique specified in the nutrition labeling regulation (Jeon, 1995).

### Minerals analysis

For determining metals, the ash obtained by incineration of the biomass was dissolved in concentrated hydrochloric acid and filtered, diluted to volume and read directly on the atomic absorption spectrophotometer (PERKIN-ELMER; A ANALYST 100, USA). Sodium, potassium, calcium, iron, zinc, copper and magnesium contents were determined (Tee et al., 1996).

### Preparation of FAME (Fatty acid methyl ester)

FAME was prepared from the extracted lipids by transmethylation with 5% anhydrous methanolic HCl crude fatty acid sample containing butyl hydroxy toluene (BHT) and was evaporated to dryness under nitrogen gas following which 50  $\mu$ l of the sample was mixed with 300  $\mu$ l n-hexane. 30  $\mu$ l of the mixture was evaporated to dryness under nitrogen gas. 1 ml methanolic hydrochloric acid and 1 ml toluene were added to the mixture and refluxed on a dry block for 2 h at 100°C. The sample was then allowed to cool at room temperature. 1 ml of distilled water and 1 ml of n-hexane was added to the sample, mixed and centrifuged at 300 rpm for 10 min. The lower layer was taken out and the procedure was repeated a few times until the soapy remnants were eliminated. Anhydrous sodium sulphate was added to the layer to eliminate water content. FAME obtained was kept at -20°C until ready for gas chromatography (GC) analysis (Hamida, 2007).

### GC analysis

FAME standard and samples were injected into GC (SHIMADZU GC 2010, JAPAN). The column temperature was maintained at 50°C for 5 min after which it was raised to 170°C at 10°C per min. Finally, the temperature was raised to 245°C at a rate of 2°C per min and kept for 2 min. Total time for GC was 56.5 min. Based on the chromatogram, the composition of fatty acid samples can be evaluated by comparing the retention time of each peak and its area with the standard. (Hamida, 2007)

### Vitamin assays

#### Vitamin A

The method described by Tee et al. (1996) was used for vitamin A extraction. 10 g of sample were weighed, 40 ml of 95% ethanol and 10 ml of KOH were added and then refluxed for 30 min. The sample was extracted with 50 ml of n-hexane three times and all the hexane extracts (upper layer) were collected, washed with distilled water and filtered through sodium sulphate anhydrous ( $\text{Na}_2\text{SO}_4$ ). The solvent was evaporated to dryness under nitrogen. Vitamin A was chromatographed using a 150 x 4.6 mm RP 18 e 5 $\mu$ m LiChroCART column (MERCK, USA) and a mobile phase of acetonitrile-isopropanol-methanol-water (39:52:5:4) with a flow rate of 1 ml  $\text{min}^{-1}$ . The vitamin was measured by UV detection at 325 nm.

#### Vitamin C (ascorbic acid)

Vitamin C was extracted according to Abdulnabi et al. (1997). 10 g of sample were mixed with 50 ml of 2% metaphosphoric acid and transferred to a conical flask. Following mechanical shaking for 15 min, the mixture was filtered through filter paper to obtain clear extracts, which were kept at -20°C until analysis. Vitamin C was chromatographed using a 250 x 4.6 mm C-18 5 $\mu$ m Zorbax column (AGILENT TECHNOLOGY, USA) and a mobile phase of 0.1 M

$\text{KH}_2\text{PO}_4$ -methanol-TBAOH (97:3:0.05) at a flow rate of 1 ml  $\text{min}^{-1}$ . The vitamin was measured by UV detection at 354 nm.

### Vitamin E

The method was according to that reported by Shin and Godber (1993). 500 mg of sample was placed in a 15 ml test tube with 5 ml ethanol and 0.1g ascorbic acid. The test tube was placed in an 80°C water bath for 10 min, after which 0.15 ml of 80% KOH was added. The sample was saponified for 10 min at 80°C, placed in an ice bath and 5 ml water and 5 ml hexane were added. The mixture was centrifuged at 120 x g for 1 min and the upper layer was collected. Extraction of the sample with 5 ml hexane was repeated twice. The pooled hexane layer was washed three times with 5 ml of water, filtered through anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) and then evaporated to dryness under nitrogen. Vitamin E was chromatographed using a 250 x 4.6 mm Allsphere silica 5  $\mu$ m Alltech column (ALLTECH ASSOCIATES, INC. ILLINOIS, USA) and a mobile phase of hexane: isopropanol (95:5) with a flow rate of 1.5 mL  $\text{min}^{-1}$ . The vitamin was measured by fluorescence detection, excitation at 294 nm and emission at 330 nm

### Statistical analysis

All analyses were performed in triplicate. All data were presented as mean values  $\pm$  S.D. and the mean values were analyzed by t-test ( $p < 0.05$ ) to detect significant differences among different culture conditions of CV.

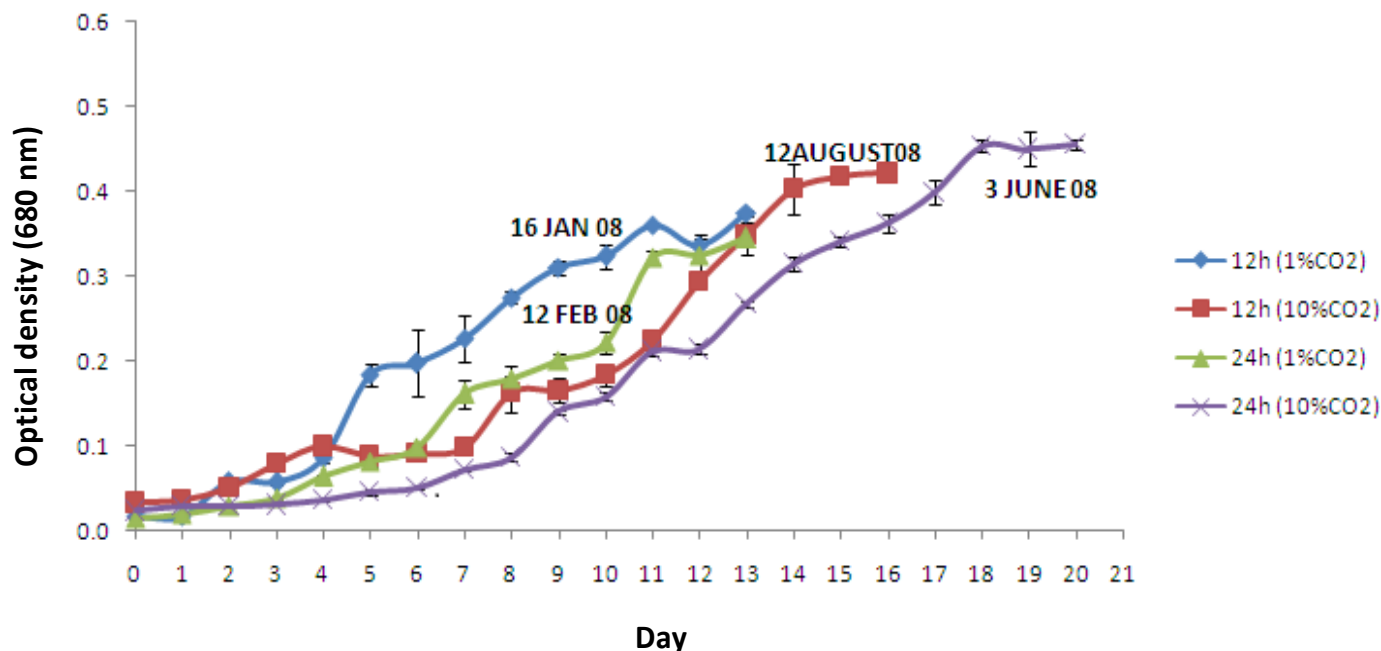
## RESULTS AND DISCUSSION

### Growth conditions

Figure 1 shows that CV cultured under 24 h light and 10%  $\text{CO}_2$  produced the best growth rate when compared with other culture conditions: 12 h light (1 and 10%  $\text{CO}_2$ ) and 24 h light (1%  $\text{CO}_2$ ). Our finding is in agreement with Tsuzuki et al. (1990) whereby CV grown in 2%  $\text{CO}_2$  produced better growth rate compared to low  $\text{CO}_2$  condition. *C. vulgaris* cultured under 24 h light and 10%  $\text{CO}_2$  gave better yield since the stationary phase was reached later (21 days) compared to other culture conditions in which the stationary phase was reached earlier (10 to 15 days). The yield of freeze dried *C. vulgaris* grown in 24 h light and 10%  $\text{CO}_2$  was 250 g versus 150 to 175 g for the other culture conditions.

### Proximate analysis

Due to the different culture conditions that is, total illumination and amount of  $\text{CO}_2$ , variation in nutritional content is expected. This can be investigated by determining their nutrient composition as shown in Table 1. Total ash values varied between 5.16 and 12.98 g/100 g. Similar values have been reported for other freshwater algae which show lower value, for example, 6 to 15 g in *Scenedesmus* and *Spirulina* (Becker and Venkataraman, 1982). The protein content was quite high in CV in all



**Figure 1.** Effect of CO<sub>2</sub> concentration and duration of illumination on the growth rate of *Chlorella vulgaris*.

**Table 1.** Proximate composition (g/100g) in *Chlorella vulgaris* (mean  $\pm$  SD).

Culture condition	Moisture	Ash	Protein	Lipid	Carbohydrate	Total dietary fiber
12 h 1% CO <sub>2</sub>	2.67 $\pm$ 1.15 <sup>a</sup>	12.98 $\pm$ 0.04 <sup>a</sup>	42.55 $\pm$ 0.18 <sup>a</sup>	1.00 $\pm$ 0.00 <sup>a</sup>	41.12 $\pm$ 1.01 <sup>a</sup>	16.37 $\pm$ 2.59 <sup>a</sup>
12 h 10% CO <sub>2</sub>	4.17 $\pm$ 0.40 <sup>a</sup>	5.16 $\pm$ 0.03 <sup>b</sup>	32.86 $\pm$ 8.03 <sup>a</sup>	1.59 $\pm$ 0.02 <sup>b</sup>	56.22 $\pm$ 8.05 <sup>b</sup>	19.98 $\pm$ 1.10
24 h 1% CO <sub>2</sub>	5.06 $\pm$ 0.18 <sup>b</sup>	8.62 $\pm$ 0.07 <sup>b</sup>	25.50 $\pm$ 4.47 <sup>b</sup>	1.11 $\pm$ 0.02 <sup>b</sup>	59.71 $\pm$ 4.62 <sup>b</sup>	25.95 $\pm$ 0.85 <sup>b</sup>
24 h 10% CO <sub>2</sub>	9.95 $\pm$ 0.07 <sup>c</sup>	6.87 $\pm$ 0.03 <sup>a</sup>	48.19 $\pm$ 1.33 <sup>c</sup>	5.60 $\pm$ 0.06 <sup>a</sup>	29.85 $\pm$ 1.17 <sup>a</sup>	17.06 $\pm$ 0.58 <sup>a</sup>

<sup>a,b,c</sup>, Comparison between culture condition. Different superscripts indicate significant differences at  $p < 0.05$  by t-test.

culture conditions, which is comparable with most microalgae, such as *Enantiocladia duperrehyi*, *Amansia multifida*, *Hypnea musciformis*, *Solieria filiformis* and *Bryothamnion seaforthii* (Benevides et al., 1998). CV cultured under 24 h light and 10% CO<sub>2</sub> showed significantly higher amount of moisture, protein and lipid values when compared to other culture conditions. Due to the low fat content when compared with ten Brazilian marine algae, CV has low caloric value, which makes it an interesting healthy food (Benevides et al., 1998). According to Benevides et al. (1998), divergence based on chemical composition is possibly due to environmental factors (salinity, temperature, light intensity and nutrient concentrations). Zavodnik (1987) observed intraspecific variations in the rate of production and chemical composition of the marine algae *Ulva rigida* and *Porphyra leucosticta*, which was dependent on growth habitats and environmental conditions. Similar results due to seasonal alterations have been reported for a number of macrophytic algae (Durako and Dawes, 1980).

## Minerals

Mineral components of CV are given in Table 2. Although CV cultured under 12 h light and 1% CO<sub>2</sub> had lower nutritional values, it contained the highest amount of minerals compared to other culture conditions. This is in correlation with the higher amount of ash as evident from Table 1. This observation is in agreement with a previous study where it was reported that legumes and cowpea had the highest content of ash and also the highest concentrations of K, Mg and P (Iqbal et al., 2006). As seen from the table, CV is a good source of Ca, K, Mg, and Zn. These minerals have many functions in human; K is associated with fluid balance and volume, carbohydrate metabolism, protein synthesis and nerve impulses; Mg is important in the nervous activity and muscle contraction (Diehl, 2002). Zinc is an essential component of enzymes which participate in many metabolic processes including synthesis of carbohydrate, lipid, and protein synthesis and it is also a cofactor of

**Table 2.** Mineral element content (mg/100g) in *C. vulgaris* (mean  $\pm$  SD).

Culture condition	Ca	Mg	Zn	Na	K	Cu	Fe
CV 12 h 1%CO <sub>2</sub>	1425 $\pm$ 10.3 <sup>a</sup>	851 $\pm$ 9.39 <sup>a</sup>	293 $\pm$ 4.48 <sup>a</sup>	101 $\pm$ 2.75 <sup>a</sup>	1197 $\pm$ 17.02 <sup>a</sup>	48.4 $\pm$ 27.90	82.2 $\pm$ 37.63 <sup>a</sup>
CV 12 h 10%CO <sub>2</sub>	106 $\pm$ 2.78 <sup>b</sup>	282 $\pm$ 21.01 <sup>b</sup>	10.2 $\pm$ 2.89 <sup>b</sup>	129 $\pm$ 9.25 <sup>b</sup>	986 $\pm$ 38.07 <sup>b</sup>	4.88 $\pm$ 0.19 <sup>a</sup>	147 $\pm$ 1.72 <sup>b</sup>
CV 24 h 1%CO <sub>2</sub>	535 $\pm$ 28.1 <sup>b</sup>	578 $\pm$ 12.60 <sup>c</sup>	102 $\pm$ 3.20 <sup>b</sup>	96.3 $\pm$ 3.50 <sup>a</sup>	784 $\pm$ 12.10 <sup>b</sup>	26.1 $\pm$ 5.80	281 $\pm$ 34.90 <sup>b</sup>
CV 24 h 10%CO <sub>2</sub>	278 $\pm$ 5.63 <sup>a</sup>	286 $\pm$ 13.71 <sup>b</sup>	59.2 $\pm$ 0.76 <sup>a</sup>	82.5 $\pm$ 5.68 <sup>c</sup>	527 $\pm$ 9.75 <sup>a</sup>	25.6 $\pm$ 4.89 <sup>b</sup>	78.8 $\pm$ 12.38 <sup>a</sup>

<sup>a, b, c</sup>, Comparison between culture condition. Different superscripts indicate significant differences at  $p < 0.05$  by t-test.

**Table 3.** Contents of vitamins in *Chlorella vulgaris*.

Culture condition	Retinol ( $\mu$ g/g)	Vitamin C (mg/g)	Vitamin E (mg/g)					Total Vitamin E
			$\alpha$ -tocopherol	$\alpha$ -tocotrienol	$\gamma$ -tocopherol	$\gamma$ -tocotrienol	$\delta$ -tocotrienol	
12 h 1%CO <sub>2</sub>	96.08 $\pm$ 13.38	0.24 $\pm$ 0.04	18.31 $\pm$ 1.60	0.61 $\pm$ 0.09 <sup>b</sup>	7.48 $\pm$ 1.21 <sup>a</sup>	0.10 $\pm$ 0.02 <sup>a</sup>	0.10 $\pm$ 0.01 <sup>a</sup>	26.60 $\pm$ 0.42 <sup>a</sup>
12 h 10%CO <sub>2</sub>	100.29 $\pm$ 18.35	0.11 $\pm$ 0.03	16.68 $\pm$ 1.97	0.46 $\pm$ 0.10	3.35 $\pm$ 0.06 <sup>b</sup>	0.10 $\pm$ 0.02	0.09 $\pm$ 0.02	20.67 $\pm$ 2.13 <sup>b</sup>
24 h 1%CO <sub>2</sub>	110.81 $\pm$ 27.42	0.17 $\pm$ 0.01	15.53 $\pm$ 0.96	0.27 $\pm$ 0.05 <sup>a</sup>	2.93 $\pm$ 0.34 <sup>b</sup>	0.04 $\pm$ 0.01 <sup>b</sup>	0.06 $\pm$ 0.00 <sup>b</sup>	18.83 $\pm$ 1.33 <sup>c</sup>
24 h 10%CO <sub>2</sub>	132.15 $\pm$ 13.98	0.39 $\pm$ 0.11	20.94 $\pm$ 3.54	0.47 $\pm$ 0.01 <sup>b</sup>	6.17 $\pm$ 0.41 <sup>a</sup>	0.10 $\pm$ 0.07	0.19 $\pm$ 0.23	27.87 $\pm$ 4.00 <sup>b</sup>

<sup>a, b, c</sup>, Comparison between culture condition. Different superscripts indicate significant differences at  $p < 0.05$  by t-Test.

the superoxide dismutase enzyme, which is involved in protection against oxidative processes (Mann and Truswell, 2009). A low Na:K ratio makes CV a unique food that mimics intracellular fluid.

## Vitamins

Table 3 shows that *C. vulgaris* cultured under 24 h light with 10% CO<sub>2</sub> contained more retinol than in other culture condition (24 h light with 10% CO<sub>2</sub>; 132.15  $\mu$ g/g compared with other culture condition; 96.08 to 110.81  $\mu$ g/g). Vitamin A, or retinol, is an essential nutrient for all mammalian species since it cannot be synthesized within the body. The amount of vitamin C was significantly greater in CV cultured under 24 h light with 10% CO<sub>2</sub> compared to other culture conditions. L-

ascorbic acid (LAA: vitamin C) is a water-soluble vitamin essential for the growth and nutrition of human and other animals. It is involved in hydroxylation processes in collagen synthesis, making it an essential nutrient in bone formation (Assunção and Mercadante, 2003). Vitamin E has physiological importance because it controls the functions of muscles and glands, and stimulates blood circulation (Salvador et al., 1998). The major form of vitamin E found in CV is  $\alpha$ -tocopherol, which varies between 15.53 and 20.94 mg/g. The highest concentration of total vitamin E was 27.87 mg/g in CV cultured under 24 h with 10% CO<sub>2</sub>. The results show that culture conditions can have a significant impact on vitamin composition. According to Abdunabi et al. (1997), some environmental and varietal factors can initiate unfavourable changes in the chemical composition of plant products. Brown et al. (1999)

also found that the content of most of the vitamins in *Nannochloropsis* sp. changed under the different light and culture conditions. Other factors that could contribute to differences between the studies include protocols for harvesting (centrifugation or filtration), processing and storage (freeze-dried or frozen at  $-20^{\circ}\text{C}$  or lower), extraction and analysis.

## Fatty acid composition

CO<sub>2</sub> concentration during growth gave various effects on *C. vulgaris*. It was found in this study that alterations in the composition of the fatty acids were actually dependent on the CO<sub>2</sub> concentration during growth and especially was more pronounced in C18 (Table 4) as was also observed by Tsuzuki et al. (1990). The amount of

**Table 4.** Percent composition of fatty acids in *Chlorella vulgaris* with different culture methods.

Fatty acids	12 h light		24 h light	
	1% CO <sub>2</sub>	10% CO <sub>2</sub>	1% CO <sub>2</sub>	10% CO <sub>2</sub>
C15:1	3.75 ± 0.00 <sup>b</sup>	ND	4.84 ± 0.13 <sup>a</sup>	1.72 ± 0.08 <sup>b</sup>
C16:0	16.33 ± 0.31 <sup>a</sup>	25.17 ± 0.06 <sup>b</sup>	15.65 ± 0.69 <sup>a</sup>	22.54 ± 0.01 <sup>c</sup>
C16:1	ND	ND	ND	2.64 ± 0.16
C17:0	9.19 ± 0.80	ND	ND	9.32 ± 0.23
C18:0	4.52 ± 0.06	ND	6.37 ± 1.08 <sup>a</sup>	1.17 ± 0.11 <sup>b</sup>
C18:1n9c	11.17 ± 0.27 <sup>a</sup>	17.63 ± 0.59 <sup>b</sup>	7.11 ± 0.38 <sup>b</sup>	13.04 ± 0.33 <sup>a</sup>
C18:2n6c	16.16 ± 0.04 <sup>a</sup>	29.68 ± 1.46 <sup>b</sup>	16.74 ± 0.30 <sup>a</sup>	26.24 ± 0.92 <sup>b</sup>
C18:3n3	21.07 ± 0.33 <sup>a</sup>	27.53 ± 0.79 <sup>b</sup>	26.94 ± 1.±39 <sup>b</sup>	16.87 ± 0.55 <sup>a</sup>
C20:0	17.01 ± 1.57	ND	22.37 ± 3.11	ND

Values are mean ± SD of n= 3 samples. a b c– Comparison between cultures, Different superscripts indicate significant differences at p<0.05 by t-Test. ND = not detectable.

C18:1, C18:2 C18:3 increased in high CO<sub>2</sub> concentrations under both culture conditions (12 and 24 h lighting). The synthesis of higher chain length fatty acid, (C-20) however seemed to be affected by the higher CO<sub>2</sub> concentration. The degree of unsaturation of C18 was enhanced under low CO<sub>2</sub> concentration compared to high CO<sub>2</sub> at both 12 and 24 h of light. This was similar to the findings of Tsuzuki et al. (1990) who found that the degree of unsaturation of 18:2 in *C. vulgaris* was higher in low CO<sub>2</sub> condition than in enriched CO<sub>2</sub> (2%) cells.

In conclusion, CV grew well under 24 h illuminations (12 h natural sunlight during the day and 12 h 40 W fluorescent light at night) under 10% CO<sub>2</sub> with optimum nutritional composition. Fatty acid composition and the degree of unsaturation were dependent on the amount of CO<sub>2</sub> in the culture. It is thus possible to modify the nutritional contents of CV by varying culture conditions.

## Abbreviations

**CV**, *Chlorella vulgaris*; **GC-MS**, gas chromatography-mass spectrometry; **CGF**, chlorella growth factor; **UMACC**, university of Malaya algae culture collection; **BBM**, bold's basal medium; **TDF**, total dietary fiber; **FAME**, fatty acid methyl ester; **BHT**, butyl hydroxy toluene; **GC**, gas chromatography; **UV**, ultraviolet-visible; **LAA**, L-ascorbic acid.

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