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Lipid Production of Marine Green Microalgae *Chlorella protothecoides* BUUC1601 by Using Spent Coffee Grounds Hydrolysate

Rachanimuk Hiransuchalert[a], Nisa Siranonthana[b], Nuttapon Chedtaisong[c], Pakawan Setthamongkol[c], Yutthaya Yuyen[d], Janjarus Watanachote[b], Paveena Tapaneeyaworawong[e, f], Nongnuch Rungsawang[g] & Maliwan Kutako[c]*

[a] *Faculty of Science, Burapha University, Chonburi, 20131 Thailand*
[b] *Institute of Marine Science, Burapha University, Chonburi, 20131 Thailand*
[c] *Faculty of Marine Technology, Burapha University, Chanthaburi Campus, Chanthaburi, 22170 Thailand*
[d] *Faculty of Science and Technology, Suan Dusit University, Bangkok, 10300 Thailand*
[e] *National Center for Genetic Engineering and Biotechnology, Pathum Thani, 12120 Thailand*
[f] *Center of Excellence for Marine Biotechnology, Chulalongkorn University, Bangkok, 10330 Thailand*
[g] *Research and Development Institute, Suan Dusit University, Bangkok, 10300 Thailand*

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Abstract

Spent coffee grounds are an organic waste that can be used as a source of microbial organic carbon. In this research, coffee grounds were hydrolyzed into a solution called spent coffee grounds hydrolysate (SCGH) using concentrated sulfuric acid. Then, the marine green microalgae, *C. protothecoides* BUUC1601, was cultured using SCGH. Growth performance and lipid accumulation of the microalgae were evaluated. The microalgae were cultured using a standard F/2 medium without and with SCGH added in the range of 2.5-15% of culture media (v/v). It was found that the microalgae had similar growth performance and biomass yield, i.e., the specific growth rate was in the range of 0.87-1.12 day^{-1} and the biomass yield was in the range of 0.05-0.08 g DW/L/day. Microalgae cultivation using F/2 with SCGH had an effect on lipid accumulation. It was found that using SCGH at 15% of the total volume resulted in an increase in the amount of lipid accumulation up to 66.03% of the dry weight. Of these, it was 2.89 times higher than the lipid content of microalgae cultured with no SCGH added. The content of monounsaturated fatty acids ranged from 46.15% to 46.53% and polyunsaturated fatty acids ranged from 32.40% to 34.62% of the total fatty acid content. Oleic acid (C18:1n9), an omega-9 fatty acid, was found to be the most abundant, accounting for over 30% of the total fatty acid content. In contrast, the omega-6 fatty acids linoleic acid (C18:2n6) and gamma-linolenic acid (C18:3n6) were found to be lower, accounting for 25.99%-27.20% and 6.03%-7.01% of the total fatty acid content, respectively. The omega-3 fatty acid such as alpha-linolenic acid (C18:3n3)

* Corresponding Author
e-mail: maliwan@buu.ac.th

was found at 4.20% in microalgae cultured using standard F/2 medium without SCGH, which was higher than in microalgae cultured with the addition of SCGH. Therefore, the addition of SCGH at a concentration of 2.5%-15% (v/v) can be used to cultivate *C. protothecoides* BUUC1601 for the lipid production with high unsaturated fatty acid content, which has the potential to be used in both aquaculture and functional food supplementation.

Introduction

Coffee drinks are popular all over the world and coffee consumption is forecasted to increase. Coffee consumption is in the form of instant coffee from the industrial sector, and fresh coffee from both households and coffee shops. Both types of coffee, instant coffee and fresh coffee, require roasted coffee beans, grinding and extraction with hot water (Cruz-Lopes et al., 2017). The production of instant coffee and fresh coffee therefore has a waste product in the form of spent coffee grounds (SCG), which increases with the demand of coffee consumption. However, coffee grounds are organic compounds, if not disposed of properly may be contaminated and have a negative impact on the environment (Ballesteros et al., 2014). Coffee grounds contain lignocellulose which is the main component such as cellulose, hemicellulose and lignin at 10.78%, 28.36% and 10.72% by weight, respectively. In addition, SCG contain protein, ash and tannin at 9.28%, 1.8% and 30.36% by weight, respectively. Thus, coffee grounds still remain as useful biological compounds especially in the form of insoluble compounds such as sugars that are composed of cellulose and hemicellulose i.e. glucose, galactose, mannose and arabinose (Cruz-Lopes et al., 2017; Scully et al., 2016). SCG can be used in the form of solid coffee grounds directly such as fertilizer because of the good water holding capacity and rich in organic matter and trace elements. Moreover, SCG also can be used as spent coffee grounds hydrolysate (SCGH). Which is a liquid form of the digestion of coffee grounds (hydrolysis) by using chemicals such as sulfuric acid or enzymes i.e. mannanase, endoglucanase, exoglucanase, xylanase and pectinase. Therefore, SCGH is rich in sugars that can be used in related fields such as biofuel production, amino acid and enzyme production and microorganism's biomass production as well arabinose (Scully et al., 2016; Jooste et al., 2013; Hudeckova et al., 2018).

Chlorella protothecoides is the marine green microalgae that has a high nutritional value and is able to grow in autotrophic, mixotrophic and heterotrophic conditions. In autotrophic growth conditions, microalgae can produce their own food through photosynthesis by using carbon dioxide gas as a carbon source. While in mixotrophic and heterotrophic growth conditions, microalgae use carbon sources in the form of organic substances such as sugars, fatty acids and acetate. For mixotrophic growth, microalgae required light during the culture period in contrast to the heterotrophic growth where microalgae can grow without light (Heredia-Arroyo et al., 2010). It has been reported that cultured *C. protothecoides* in mixotrophic and heterotrophic growth conditions can have an effect on lipid accumulation better than cultured under autotrophic growth conditions. Therefore, unsaturated fatty acids in *C. protothecoides* will increase such as oleic acid (C18:1n9), linoleic acid (C18:2n6) and gamma-linolenic acid (C18:3n6). These polyunsaturated fatty acids are very important in biofuel, nutraceutical and pharmaceutical (Da Rosa et al., 2020).

In this research, SCG was collected from coffee shops in Mueang District, Chanthaburi Province, Thailand. The SCG was hydrolyzed with concentrated acid to prepare SCGH for lipid production of *C. protothecoides* BUUC1601. Microalgae can be used in related fields such as aquaculture, functional food and biofuel production. In addition, the result of this experiment provides suggestions for usage of SCG which can be created from the waste from coffee shops, domestic and coffee industry to be more functional.

Materials and methods

1. Microalgae strain

Marine green microalgae *C. protothecoides* BUUC1601 in this experiment was isolated from the coastal area in Chanthaburi Province (Phirulpawadee, 2016). The microalgae inoculum was maintained at the Marine Biotechnology Research Unit, Faculty of Marine Technology, Burapha University. It was grown in standard F/2 medium (Guillard, 1973) with a salinity at

30 psu and under constant light using cool-white, fluorescent lamps at 80 µM/m²/s.

2. Culture medium

Standard F/2 medium (Guillard, 1973) was used for this research. The medium consisted of the following components (per liter of seawater): 75 mg $NaNO_3$, 5 mg $Na_2HPO_4.H_2O$, 30 mg $NaSiO_3.9H_2O$, 0.436 mg Na_2 EDTA, 0.315 mg $FeCl_3.6\ H_2O$, 0.01 mg $CuSO_4.5H_2O$, 0.022 mg, ZnSO4.7H_2O, 0.01 mg $CoCl_2.6H_2O$, 0.18 mg $MnCl_2.4H_2O$, 0.006 mg $Na_2MoO_4.2H_2O$, 0.2 mg Thiamine-HCl, 1 mg Biotin and 1 mg Cyanocobalamin.

3. SCG source

Arabica coffee grounds were obtained by courtesy of a coffee shop in Muang District, Chanthaburi Province, Thailand and used throughout this research.

4. SCGH preparation

The SCG was baked at 60˚C for 48 hr. After baking, it was digested with 98% concentrated sulfuric acid using SCG to sulfuric acid ratio of 1:8 w/v. The mixture was gently stirred continuously for 30 min and during digestion, the SCG was allowed to cool. Then, sodium hydroxide (0.5 M) was added to adjust the pH to 7.0 (Sime et al., 2017). SCG were separated by filtering through a nylon fabric filter with a pore size of 15 µm and glass fiber filter Whatman® GF/C (0.45 µm), respectively. The SCGH was used for further experimentation.

5. *C. protothecoides* BUUC1601 cultivation using SCGH

Natural sea water was filtered with a 20 µm polyester fabric filter, then chlorine was added to a final concentration at 5 mg/L and aerate for 24 hr. The standard Gillard's F/2 medium was prepared by using seawater which was provided from the previous step. The F/2 medium was then autoclaved at 121°C and 15 psi for 20 min. *C. protothecoides* BUUC1601 was cultured in a 250 mL Erlenmeyer flask with 100 mL culture medium. Then divided into 5 experimental sets i.e., standard Gillard's F/2 culture medium, F/2+2.5% SCGH (v/v), F/2+5% SCGH, F/2+10% SCGH and F/2+15% SCGH, respectively. Inoculum of microalgae was added into the culture medium with 10% (v/v). The cultures were placed in a room at 26°C with 80 µM/m²/s fluorescent lamp for 16 days. Shaking the culture by hand was done once a day for 3 min.

6. Analytical techniques

6.1 Cell counting

The cell count of microalgae was determined using a hemocytometer. Then, cell density and specific growth rate (μ) were calculated by using Eq. 1 (Roleda et al., 2013).

$$\mu = \frac{\ln(N_2 - N_1)}{t_2 - t_1} \quad (1)$$

When N_1 and N_2 as cell density (cells/mL) at time t_1 and t_2 (day), respectively

6.2 Dry weight

C. protothecoides BUUC1601 was cultured for triplicate. Microalgae was grown in standard F/2 medium with a salinity at 30 psu and under constant light using cool-white, fluorescent lamps at 80 µM/m²/s for 12 days. Culture was collected for cell counting and dry weight analysis every 2 days. Biomass of microalgae was filtered on dried GF/C filter paper, then rinse with distilled water and kept at 105°C until a stable weight (Ratomski & Hawrot-Paw, 2021). Filter paper was weighed and calculated of microalgae dry weight (g/L). XY-distributed graph was created, which X-axis=cell density (cells/mL) and Y-axis=microalgae dry weight (g/L). A linear equation was created as y=0.0058x +0.3992 (R^2=0.9529) for assessed dry weight in this experiment. Biomass productivity (*P*) in g/L/day was calculated using Eq. 2 (Ratha et al., 2016).

$$P = \frac{(N_2 - N_1)}{t_2 - t_1} \quad (2)$$

When N_1 and N_2 as microalgae dry weight at time t_1 and t_2, respectively

6.3 Total sugar content in the culture medium

The phenol-sulfuric acid method (Dubois et al., 1956) was used to determine total sugar content in the culture medium. First, the culture medium was filtered through GF/C filter paper. Then, 5.0 mL of filtered culture medium was pipetted and 5.2 mL concentred sulfuric acid and 0.5 mL of 5% Phenol were added. Then mixed to be homogeneous and incubated in a fume hood for 30 min. The absorbance was measured with a wavelength at 490 nm and calculated for the total sugar content (mg/L) by using the linear equation from the standard curve of the glucose solution.

6.4 Proximate composition analysis

The AOAC (1997) method was used to determine the proximate composition of SCG. The moisture content was determined by drying SCG in an oven at 105°C for 6 hr. The ash content was estimated by heating SCG in a muffle furnace at 550°C for 6 hr. Protein was determined

using the Kjeldahl's method. Lipids were extracted from SCG using petroleum ether and conducted in a Soxhlet extractor. The fiber content was analyzed using SCG that had been extracted oil. The grounds were boiled with 1.25% sulfuric acid and washed with boiled water, then boiled with 1.25% sodium hydroxide and washed again with boiled water. The mixture was filtered through a plankton net and the residue was baked at 100°C for 2 hr and then burned at 550°C for 5 hr. For carbohydrates, it was calculated using Eq. 3:

Carbohydrate (%) = 100 – ((Moisture (%) + Protein (%) + Lipid (%) + Fiber (%) + Ash (%)) (3)

6.5 Lipid extraction

At day 16 of the experiment, microalgae were collected by centrifugation at 10,000 rpm for 5 min. Next, it was important to discard the culture medium and rinse again with distilled water. Cell was dried by lyophilization method. The 50 mg of dried cells were extracted for lipids according to the method of Folch et al. (1957) by using chloroform: methanol (2:1). The lipid solution was evaporated by a rotary evaporator to eliminate mixed solvents. Next, they were sprayed with nitrogen gas, weighed for lipid determination and reported in % in dry weight.

6.6 Fatty acid analysis

Lipid extracted was dissolved with solvent, chloroform: methanol mixed with butylated hydroxytoluene (BHT) and transesterification by modified Christie's (2003) method. The solution was evaporated and dried with nitrogen gas, and the fatty acids were dissolved with 1 mL of n-hexane. The fatty acid solution was analyzed by gas chromatography (model 7890A, Agilent) with Flame Ionization Detector (FID), column model 19091N-133, HP-INNOWAX (Agilent). One µL sample was injected in split (5:1), flow rate of helium gas 1.1 mL/min, injection point temperature 240°C and detector temperature 260°C, which started at 35°C and maintained for 0.5 min. The temperature was then increased to 170°C at a rate of 3°C/min, maintained for 3 min, and the temperature was increased to 240°C at a rate of 2°C/min, maintained for 3 min. The fatty acid classification was compared with the retention time of Standardized Fatty Acids 37 Component FAME Mix (Supelco).

7. Statistical analysis

The average specific growth, cell yield, lipid yield of microalgae, and total sugar content in each experimental unit were calculated. The data were then subjected to One-Way Analysis of Variance (One-Way ANOVA) to analyze variance and compared differences between the means of experimental sets using Duncan's tests at a 99% confidence level. The statistical analysis was conducted using SPSS software (version 15; SPSS Inc.; Chicago, IL, USA).

Results and discussion

1. SCG and SCGH

The moisture content of SCG in the experiment was 6.90±0.03%. While the dry SCG contained 58.04% of carbohydrates, followed by fiber and protein at 19.12 and 13.94% in dry weight, respectively. The percentage of ash and lipid content was only 1.35 and 0.66%, respectively (Table 1). Dried SCG was digested using concentrated sulfuric acid at a 1:8 ratio while stirring constantly. Cooling was employed during digestion to reduce the severity of the reaction. Then, the pH value was adjusted to 7.0 and large particles were filtered to obtain SCGH. The total sugar content of SCGH was 69.21±1.94 mg/L.

SCG containing carbohydrates at 58.04% of dry weight were digested with strong acids, producing sugar products. Mussatto et al. (2011) reported that SCG contains approximately 50% of polysaccharides in dry weight. When SCG was digested by hydrolysis with sulfuric acid, it was found that SCGH consisted of mannose, galactose and arabinose at 21.2%, 13.8% and 1.7% by weight, respectively. The result suggests that SCGH may be able to be used as an organic carbon source for microalgae cultivation such as *Chlorella* and *Spirulina.* Microalgae can use organic carbon together with their food production from photosynthetic or mixotrophic (Melo et al., 2018). Therefore, in the mixotrophic cultivation, agricultural waste or wastewater from the processing of agricultural products in both solid and liquid form were used as ingredients of culture medium (Silkina et al., 2019).

Table 1 Chemical composition in SCG (mean percent ± SD) used in this research

Chemical composition	% Dry weight
Moisture content	6.90±0.03
Protein	13.94±0.18
Lipid	0.66±0.01
Carbohydrate	58.04±0.25
Ash	1.35±0.01
Fiber	19.12±0.08

2. *C. protothecoides* BUUC1601 Growth

Fig. 1 below, indicates that the use of SCGH in the culture medium had an effect on microalgae growth similar to the standard F/2 medium in autotrophic growth conditions (without SCGH). The marine green microalgae, *C. protothecoides* BUUC1601 was grown in F/2 culture medium with varying concentrations of SCGH. It was no significant difference in the specific growth rate, maximum cell density and biomass productivity ($p > 0.05$). With mixotrophic growth, *Chlorella* cultured with F/2 medium mixed with SCGH, had a growth efficiency and biomass yield similar to a cell that was cultured with F/2 medium. The specific growth rates ranged from 0.87 to 1.12 day^{-1} and biomass yields ranged from 0.05 to 0.08 g DW/L/day ($p > 0.05$) (Table 2). When *Chlorella* was cultured in F/2 medium without the addition of SCGH, its yield was slightly higher than that of *Chlorella* cultured using SCGH. This may be due to the F/2 medium containing a sufficient nitrogen source (in the form of nitrate) for their growth. Therefore, *Chlorella* can be used to generate new cells, which the accumulation of metabolites is reduced (Rios et al., 2016). The F/2 medium with SCGH had a higher source of organic carbon in the form of sugar than the F/2 medium without SCGH (Fig. 2), but nitrogen or nitrate in culture medium will be diluted. In addition, the preparation of SCGH by using strong acid digestion and adjusting the pH with alkali to neutralize the pH of SCGH effects the other nitrogen sources such as losing the free amino acid in the spent coffee grounds. Therefore, it may have an effect on C:N ratio in the culture medium to change. Silaban et al. (2014) reported that the mixotrophic culture of *Chlorella vulgaris* with a C:N ratio at 15:1 had a good result on their growth. However, when considering the biomass yield of microalgae, it indicated that microalgae cultivation by F/2 medium with the addition of SCGH in the range of 2.5-15% can produce biomass of *C. protothecoides* BUUC1601.

The results of this experiment showed that the sugar content in SCGH did not have any significant effect on the growth of *C. protothecoides* BUUC1601. However, a decrease in the total sugar content was observed in all experimental units (Fig. 2). Therefore, adding organic carbon to culture medium may also inhibit microalgae growth as well (Rios et al., 2016). The F/2 medium containing SCGH ranged in color from pale yellow to light brown, depending on the amount of SCGH added. This variation in color is due to the presence of tannins in SCGH, which may also cause the Maillard reaction during coffee roasting. It is important to note that the color of coffee beans can vary based on the specific strain of coffee, as well as the temperature and duration of roasting (Hariyadi et al., 2020). Therefore, if high concentration of SCGH is added to the culture medium, the color of the culture media darkens. It may have an effect on light transmittance of medium lower than the F/2 medium without SCGH and may have an effect on decreasing the photosynthetic efficiency.

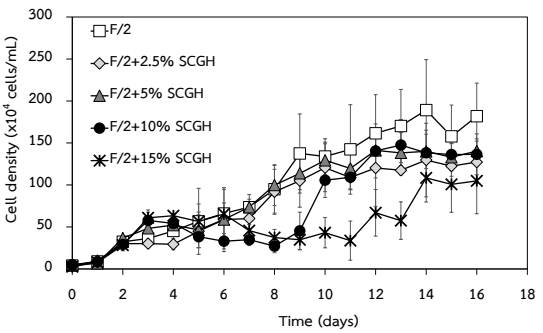

Fig. 1 Growth profiles of *C. protothecoides* BUUC1601 in each experimental unit during 16 days of culture (error bars = ± SD)

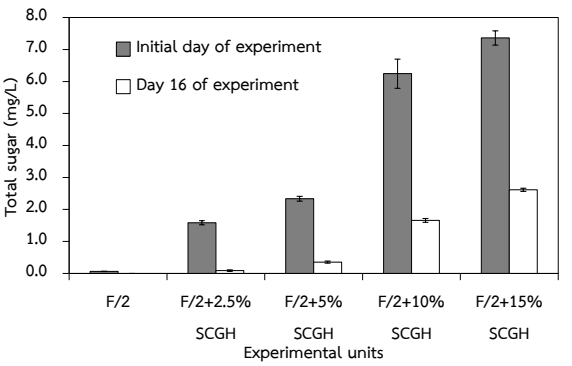

Fig. 2 Total sugar content in the culture of *C. protothecoides* BUUC1601 at initial day and the end of experiment (error bars = ± SD)

Table 2 Specific growth rate, maximum cell density and biomass productivity of *C. protothecoides* BUUC1601 (mean ± SD) cultured in F/2 culture medium supplied with different SCGH concentrations

Experimental unit	Specific growth rate^{ns} (day^{-1})	Maximum dry weight^{ns} (g DW/L)	Maximum biomass productivity^{ns} (g DW/L/day)
F/2	1.05±0.27	1.50±0.35	0.08±0.02
F/2+2.5% SCGH	1.12±0.07	1.19±0.20	0.06±0.02
F/2+5% SCGH	1.02±0.1	1.41±0.15	0.08±0.01
F/2+10% SCGH	0.87±0.20	1.26±0.17	0.06±0.01
F/2+15% SCGH	1.08±0.18	1.08±0.13	0.05±0.01

Remark: ns means no significant difference at $p > 0.05$

3. Lipid content and fatty acid composition in *C. protothecoides* BUUC1601

C. protothecoides BUUC1601 were grown in a stationary phase between days 14-16. The color of microalgae which was cultured in F/2 medium without SCGH was still green. Whereas, the microalgae cultured with F/2 medium with SCGH turned a yellowish green-yellow. The microalgae cultivated with F/2 medium by adding 15% SCGH (v/v) was yellower than the other experimental units (Fig. 3). When the cells were collected for lipid extraction, it was found that the addition of 15% SCGH had a highest lipid accumulation at 66.03±5.08% in dry weight ($p < 0.05$) (Table 3). It was shown that the microalgae culture with F/2 medium with 2.5-15% SCGH added had a higher lipid content than in F/2 medium without SCGH with 1.83 times, 2.45 times, 2.51 times and 2.89 times, respectively. Lizzul et al. (2018) reported that *C. sorokiniana* cultured in an autotrophic condition without added organic carbon had a lipid content range from 18–22%, which was similar to that of *C. protothecoides* BUUC1601 in this experiment (22.83%).

The SCGH contains various types of organic carbon such as fats, fatty acids and carbohydrates (Cruz-Lopes et al., 2017; Fei et al., 2015), which can serve as precursors for lipid production by microalgae. However, in this study, it was found that the addition of SCGH did not promote microalgae growth but changed their color from green to yellow (Fig. 3). This indicates that the microalgae may be under stress or have a low nitrogen content. SCGH contains a significant amount of organic carbon, but there is a limited amount of nitrogen that may have been lost during the process of preparing SCGH. This can affect microalgae by reducing protein production, stopping cell regeneration and increasing the accumulation of metabolites in the form of lipids or carbohydrates (Krzemin´ska et al., 2015; Ferreira et al., 2009). In addition, Converti et al. (2009) reported that factors such as inappropriate light intensity or lack of nutrients in culture media, especially nitrogen, can lead to increased lipid accumulation in microalgae. Therefore, due to limited nitrogen source *C. protothecoides* BUUC1601 was able to accumulate more lipids under the conditions of this study.

Fig. 3 Color of *C. protothecoides* BUUC1601 at the end of experiment

Table 3 Lipid content in *C. protothecoides* BUUC1601 (mean percent ± SD) cultured with different SCGH concentrations

Experimental unit	Lipid content (% in dry weight)
F/2	22.83±8.31^{c}
F/2+2.5% SCGH	41.86±10.19^{b}
F/2+5% SCGH	55.84±4.22^{ab}
F/2+10% SCGH	57.27±2.51^{ab}
F/2+15% SCGH	66.03±5.08^{a}

Remark: Values followed by different letters denote significant difference at $p < 0.05$

C. protothecoides BUUC1601 is abundant in saturated fatty acids such as myristic acid (C14:0), C15:0, palmitic acid (C16:0), stearic acid (C18:0) and arachidic acid (C20:0). When compared to cells cultured in F/2 medium without SCGH, those cultured with SCGH exhibited significantly lower amounts of C16:0 ($p < 0.05$), which accounted for up to 16.87% of total fatty acids. Ötleo & Pir (2001) reported that *Chlorella* contains palmitic acid ranging from 14.42% to 17.22%. Palmitic acid is a precursor for the synthesis of both saturated and unsaturated fatty acids and serves as the primary component of biodiesel for fuel energy production.

C. protothecoides BUUC1601 cultured with SCGH exhibited significantly higher levels of the total monounsaturated fatty acids compared to cells cultured in F/2 medium without SCGH (Table 4). The addition of SCGH at levels of 2.5-15% did not have a significant effect on the accumulation of polyunsaturated fatty acids ($p > 0.05$). Cells contained more total monounsaturated fatty acids (MUFAs) than polyunsaturated fatty acids, including myristoleic acid (C14:1), ginkgolic acid (C15:1), palmitoleic acid (C16:1) and oleic acid (C18:1n9). Oleic acid was the most abundant monounsaturated fatty acid in *C. protothecoides* BUUC1601 and in the genus *Chlorella* (Ferreira et al., 2017; Isleten-Hosoglu et al., 2012). Moreover, cells cultured in F/2 medium with SCGH (2.5-15%) had higher levels of C18:1n9 (c+t) up to 33.84-38.21% of total fatty acids, compared to cells cultured in F/2 medium without SCGH ($p<0.05$). Ferreira et al. (2017) reported that microalgae cultured under heterotrophic

conditions with organic carbon added to the medium and incubated in the dark accumulated up to 35.1% oleic acid. Although oleic acid is a non-essential fatty acid, it is an important energy fuel (Deshmukh et al., 2019).

The polyunsaturated fatty acid content of *C. protothecoides* BUUC1601 cultured with SCGH was significantly higher than cells cultured in F/2 medium without SCGH ($p < 0.05$), with a range of 32.40-34.62% of total fatty acids. Linoleic acid (C18:2n-6) was the most abundant polyunsaturated fatty acid, followed by gamma-linolenic acid (C18:3n6) and alpha-linolenic acid (C18:3n3) and the addition of SCGH had the greatest impact on the accumulation of linoleic acid, which accounted for 25.99-27.20% of total fatty acids. Cheng et al. (2013) also reported that *C. protothecoides* had high levels of C18:2 and C18:3. These fatty acids are essential for human health, with potential benefits including reducing the risk of heart disease, preventing cancer, arthritis, high blood pressure and diabetes (Kaur et al., 2014). They are also important for aquaculture, as Jardine et al. (2020) found that linoleic acid and alpha-linolenic acid can improve fish growth and survival rate. However, they are sensitive to oxidation reactions, which may affect the oxidation stability in biodiesel production.

The results of this experiment demonstrated that the addition of SCGH to F/2 medium at 2.5-15% could increase the content of certain unsaturated fatty acids in *C. protothecoides* BUUC1601, particularly oleic acid, linoleic acid, gamma-linolenic acid and alpha-linolenic acid. This may be attributed to the fact that coffee is a rich source of fatty acids. Figueiredo et al. (2015) reported that *Coffea arabica* L. cultivated in Brazil contained C18:1 in the range of 0.37-10.73%, C18:2 in the range of 36.68-41.50% and C18:3 at 0-1.64%. Therefore, the addition of SCGH to the culture medium may have contributed to the increased accumulation of fatty acids in the microalgae.

Conclusion

SCGH prepared from spent coffee grounds can be added to the F/2 medium for *C. protothecoides* BUUC1601 culture under mixotrophic conditions. SCGH helps the microalgae accumulate more lipids than the standard F/2 medium without SCGH (under autotrophic conditions). Adding SCGH at 15% (v/v) can increase the amount of lipid up to 66.03% in dry weight, which is 2.89 times more than microalgae cultured without SCGH.

Table 4 Fatty acid content (expressed as percentage of total fatty acids) in *C. protothecoides* BUUC1601 cultured with different SCGH concentrations

Fatty acid	F/2	F/2+2.5% SCGH	F/2+5% SCGH	F/2+10% SCGH	F/2+15% SCGH
SFAs					
C14:0	2.62±2.99^{a}	2.83±0.84^{a}	1.84±0.40^{a}	3.32±0.23^{a}	2.95±0.42^{a}
C15:0	1.53±1.73^{a}	1.06±0.42^{a}	0.62±0.15^{a}	0.71±0.41^{a}	0.87±0.20^{a}
C16:0	16.87±7.14^{a}	4.27±0.60^{b}	3.21±0.17^{b}	4.07±0.69^{b}	4.42±0.33^{b}
C17:0	3.01±1.08^{a}	3.79±0.21^{a}	3.35±0.28^{a}	3.21±0.14^{a}	3.28±0.05^{a}
C18:0	6.90±2.53^{a}	7.19±0.24^{a}	11.35±3.90^{a}	8.14±0.16^{a}	9.03±0.20^{a}
C20:0	0.74±0.84^{a}	0.83±0.50^{a}	0.52±0.07^{a}	0.65±0.05^{a}	0.61±0.06^{a}
C24:0	1.85±4.53	N. D.	N. D.	N. D.	N. D.
MUFAs					
C14:1	5.00±4.67^{a}	1.08±0.85^{a}	1.8±0.67^{b}	0.78±0.07^{a}	0.68±0.08^{a}
C15:1	3.19±4.04^{a}	1.82±0.92^{a}	1.25±0.16^{a}	1.67±0.57^{a}	1.67±0.21^{a}
C16:1	4.61±1.61^{b}	6.85±2.78^{a}	4.64±0.55^{ab}	5.09±0.07^{ab}	4.82±0.27^{ab}
C17:1	N. D.	0.58±0.08^{a}	0.41±0.13^{a}	0.4±0.05^{a}	0.37±0.07^{a}
C18:1n9c	21.62±5.45^{b}	31.70±1.20^{a}	34.46±1.21^{a}	34.27±0.41^{a}	32.63±0.27^{a}
C18:1n9t	2.08±0.47^{b}	2.84±1.40^{ab}	3.62±0.27^{a}	3.94±0.09^{a}	3.71±0.10^{a}
C20:1	1.14±2.78^{a}	1.30±1.63^{a}	0.35±0.06^{a}	N. D.	0.35±0.07^{a}
C22:1n9	1.00±2.45	N. D.	N. D.	N. D.	N. D.
PUFAs					
C18:2n6	17.61±5.18^{b}	27.18±0.90^{a}	25.99±1.34^{a}	26.93±0.61^{a}	27.20±0.33^{a}
C18:3n6	N. D.	6.77±0.21^{a}	6.03±0.22^{a}	6.19±0.15^{a}	7.01±0.33^{a}
C18:3n3	4.20±1.98^{a}	0.49±0.24^{b}	0.38±0.06^{b}	0.64±0.24^{b}	0.41±0.10^{b}
C20:2	2.93±7.17	N. D.	N. D.	N. D.	N. D.
C22:2	2.55±6.24	N. D.	N. D.	N. D.	N. D.
C22:6n3	0.56±1.36	N. D.	N. D.	N. D.	N. D.
Total SFAs	33.52±0.90^{a}	19.97±0.01^{b}	20.89±0.827^{b}	20.10±0.17^{b}	21.16±0.06^{b}
Total MUFAs	38.64±0.42^{b}	46.17±0.80^{a}	46.53±0.41^{a}	46.15±0.01^{a}	44.23±0.08^{a}
Total PUFAs	27.83±0.48b	34.44±0.20a	32.40±0.69a	33.76±0.42a	34.62±0.24a

Remark: N.D: Not detected, SFAs: Saturated fatty acids, MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids, Values in each row followed by different letters denote significant difference at $p < 0.05$

However, adding SCGH to the medium has an effect on the monounsaturated fatty acid content, increasing it to 46.15-46.53% and the polyunsaturated fatty acid content to 32.40-34.62% of total fatty acids. Oleic acid was found to be more than 30% of total fatty acids, while omega-6 fatty acids such as linoleic acid were found at 25.99-27.20% and gamma-linolenic acid at 6.03-7.01% of total fatty acids. The research found that cultivating the microalgae with the addition of SCGH at 2.5-15% can produce lipid in *C. protothecoides* BUUC1601, indicating its potential use in both aquaculture and as a functional food supplement.

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