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Research Article

Investigation of Growth Performance, Proximate and Fatty Acid Composition of Freshwater (*Euglena gracilis, Chlorella vulgaris*) and Marine (*Pavlova lutheri, Diacronema vlkanium*) Microalgae

Merve Sayar[1], Kamil Mert Eryalçın[1]

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ORCID IDs of the author:
M.S. 0000-0002-2628-0608;
K.M.E. 0000-0002-8336-957X

[1]Faculty of Aquatic Sciences, Department of Aquaculture and Fish Diseases, Phytoplankton-Zooplankton Culture Laboratory, Istanbul University, Istanbul, Turkiye

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Correspondence:
Kamil Mert Eryalçın
E-mail:
eryalcin@istanbul.edu.tr

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ABSTRACT

This work is focused on investigating the nutrient compositions, growth, and fatty acid composition of *Chlorella vulgaris*, *Euglena gracilis*, *Pavlova lutheri*, and *Diacronema vlkanium*, which are natural diets of bivalve, crustaceans, live prey such as rotifer, copepods, daphnia and feed ingredients in aquaculture nutrition. Microalgae culture was performed in a live feed laboratory under controlled physical and chemical conditions. The initial concentration of microalgae species was adjusted as 2×10^6 cells/mL and growth performance was calculated by Neubauer Hemocytometer daily. The maximum growth performance was detected in *Diacronema vlkanium* culture with 1.78×10^7 cells/mL. In the case of proximate composition, the highest dry matter content was found in *Pavlova lutheri* (6.21%). Freshwater microalgae species *Chlorella vulgaris* (50.5%) and *Euglena gracilis* (42.5%) had high crude protein compared to *Pavlova lutheri* and *Diacronema vlkanium*. Fatty acid compositions of microalgae were also determined. The highest EPA (C20:5n-3) content was found in *Pavlova lutheri* (6.85%) whereas arachidonic acid (C20:4n-6) and docosahexaenoic acid (C22:6n-3) contents were only found with a level of (3.32%) and (1.79%) in *Euglena gracilis*, respectively. Microalgal culture should have high biomass in a short time of culture and in this study, *E.gracilis* and *P.lutheri* showed high growth and essential nutrients gain in laboratory scale production and this result could be applied in larger volume photobioreactor.

Keywords: Microalgae, growth, fatty acids, proximate, biomass

INTRODUCTION

Microalgae contribute greatly to both the marine and freshwater food-web and they are able to synthesize inorganic matter into organic compounds such as lipids, polysaccharides and pigments (Chiu et al., 2011). They are used for live prey enrichment and feeding (Eryalçın, 2018; Eryalçın, 2019; Turcihan et al., 2021; Turcihan et al., 2022), wastewater treatment (Wollmann et al., 2019), biodiesel production (Goh et al., 2019), fish diet ingredients (Eryalçın et al., 2013; Eryalçın and Yıldız, 2015; Eryalçın et al., 2015; Camacho-Rodríguez et al., 2018; Soto-Sánchez et al., 2023) and bivalve culture (Shah et al., 2018). Microalgae must be nutritionally riched in essential biochemical compounds such as polyunsaturated fatty acids (PUFAs), highly unsaturated fatty acids (HUFAs), essential amino acids (EAA), and pigments (Raja et al., 2004; Patil et al., 2005; Patil et al., 2007; Hemaiswarya et al., 2011; Singh et al., 2015; Peltomaa et al., 2017). Moreover, they have antigonistic effects on bacterial communities in culture tanks (Spolaore et al., 2006; Neori, 2011). The first priority of microalgae culture is to get fast high biomass gain in a short time. The fast growth performance of microalgae is based on several parameters. The rapid proliferation of microalgae contributes to the high biomass in wet weight and this leads to the possible production of nutrients such as lipid, protein, carbohydrate, and pigment. For example, dinoflagellate *Crypthecodinium cohnii* can contain DHA up to 40% in dry weight that is nesessary for both growth and stress resistance at fish larval cultivation (Eryalçın et al., 2013). Nutrient contents of microalgae such as protein, lipids, and pigments can be species-specific which means each alga can contain specific nutrients (Das et al., 2012; Eryalçın, 2019; Gharajeh et al., 2020). For instance, *Nannochloropsis oculata* contains a high amount of EPA whereas dinoflagellate *Crypthecodinium cohnii* is famous for DHA. In comparison, freshwater microalgae are rich in essential 18C chain fatty acids such as linoleic (C18:2n-6), and α-linolenic acid (C18:3n-3) which are also important for freshwater fish.

Moreover, microalgae are the main energy source and substantial for enhancing the survival and growth of bivalve larvae (Parrish et al., 1998; Pazos et al., 1997; Budge et al., 2001). The nutritional value of microalgae is changed during their culture time. There are two main phases of the culture period called the exponential and stationary phases where algae should be harvested (da Silva Ferreira and Anna, 2017). Not only the culture phase but also the culture medium affects the nutritional value of microalgae biomass. These nutrient profiles consist of macronutrients (nitrogen, phosphorus, and sulphur) and micronutrients (iron, manganese, sodium molybdenum oxide, zinc, copper, and selenium) (Aslam et al., 2021; Shaaban et al., 2010). The nitrogen source of microalgae increases the growth performance and nutritional content by synthesizing large nutrient molecules like minerals and proteins (Procházková et al., 2014; Kumaran et al., 2023).

The growth performance and nutritional composition of microalgae also depend on physical and chemical parameters such as light, temperature, salinity, pH, and cultivation methods such as heterotrophic, autotrophic, and mixotrophic culture (Bashir et al., 2019; Zhao et al., 2011). Salinity and light conditions are very important in the cultivation of microalgae. For instance; Nannochloropsis sp. shows high growth performance at high salinity levels but it shows slow growth performance in heterotrophic culture (Bashir et al., 2019). In particular, autotrophic microalgae species directly affect the synthesis of biochemical substances and growth performance due to the intensity of the light (Sandnes et al., 2005). The reason is because these microalgae species use light as an energy source. As a result, biomass, proximate and fatty acid composition change depending on the light intensity. Another agent affecting microalgal growth performance, and fatty acid composition, microalgae cell metabolism, and the initial enzyme used for photosynthesis is culture temperature (Chaisutyakorn et al., 2018; Chiu et al., 2011). The increasing temperature in the culture adversely affects the fatty acid composition of microalgae. Most importantly, higher biomass gain and growth performance as well as protein and lipid contents in most microalgae are linearly related to light intensity and photoperiod such as *Chlorella vulgaris, Ankistrodesmus falcatus*, *Monoraphidium* sp., *Botryococcus braunii* (He et al., 2015; Metsoviti et al., 2019).

The other reason that affects biomass gain of microalgae depends on the growth potential of the species. As the growth performance increases, the biomass recovery rate also increases (Lau et al., 2022). The size of microalgae cells also affects their growth performance. Small-sized microalgae show higher growth performance than larger cell microalgae due to doubling time. For example, Arkronrat et al. (2016) have stated that *Nannochloropsis sp.* are smaller microalgae, and its growth performance is faster than *Tetraselmis sp.* due to its size. The unicellular freshwater eukaryote *Euglena gracilis* obtains flagellates and instead of a cell wall it has a pellicle based protein layer by a substructure of microtubules (Zhang et al., 2023). *Euglena gracilis* is rich in Paramylon which is a linear β-1,3-glucan polysaccharide polymer, antioxidants such as β-carotene, α-tocopherol and L-ascorbic acid, and PUFAs (Kottuparambil et al., 2019). The other freshwater microalgae *Chlorella vulgaris* belongs to Chlorophyceae with the thick cell walls and contains a high level of protein, minerals, vitamins, and pigments (Spínola et al., 2023). *Pavlova lutheri* is unicellular motile marine prymnesiophyte algae containing flagellate and it is known for high sterols, EPA and, DHA contents (Ahmed et al., 2015). *Diacronema vlkanium* is another marine green microalgae that belongs to the Haptophyceae family and is also rich in high levels of EPA and DHA (Fradique et al., 2013).

In this study, growth performance, proximate, and fatty acid compositions two unicellular both freshwater (*Euglena gracilis* and *Chlorella vulgaris*) and two marine microalgae species (*Pavlova lutheri*, *Diacronema vlkianum*) were investigated under laboratory conditions for biomass utilization.

MATERIAL AND METHODS

Microalgae strains and stock culture

Culture mediums f/2 and 3N-BBM-V were sterilized at 121 °C for 15 min before they were used (Guillard, 1975). Stock culture of microalgae was cultured in 50 mL test tubes to 250 mL, followed by 1-L, and 5-L erlenmayers. Microalgae were counted in each experimental flask during the experiment. The microalgae growth trial was conducted in the Phytoplankton and Zooplankton Laboratory of the Faculty of Aquatic Sciences of Istanbul University, for 32 days. Four microalgae species were obtained from CCAP (Culture collection of algae and Protozoa, Scotland) which are *Pavlova lutheri* (Strain number: CCAP940/2), *Diacronema vlkianum* (Strain number: CCAP914/1), *Euglena gracilis* (Strain number: CCAP1224/38), *Chlorella vulgaris* (Strain number: CCAP211/110).

Experimental design and growth performance

In this study, the microalgae species were cultured from the initial to 15 days under 250 mL volume. Each experimental group was studied in three replicates. The initial cell density of the microalgae species was adjusted at $2x10^6$ cells/mL for the second part of the culture experiment after the 15^{th} day culture, all volumes were inoculated into 1000 mL erlenmayers with gentle aeration till the 32nd day. This method was chosen by the same culture procedure at commercial hathcheries where first culture occured in small-scale flasks and then continuously up-scaled in larger volumes. At the end of the culture, all biomass was harvested and stored at -80 °C in the refrigerator. During culture, the growth performance of microalgae species was calculated daily with a Neubauer Hemocytometer.

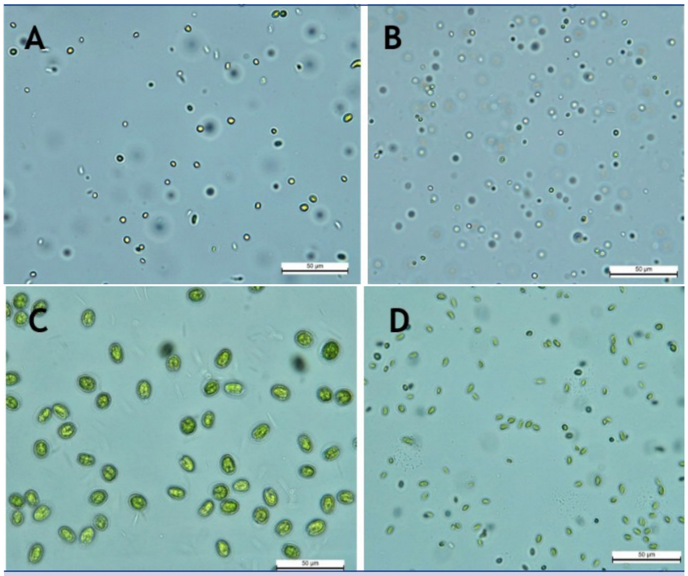

Figure 1. *Chlorella vulgaris* (A), *Diacronema vlkanium* (B), *Pavlova lutheri* (C) and *Euglena gracilis* (D).

Proximate analysis

Dry matter analysis of microalgae, the samples were first filtered using a vacuum filtration system. Vacuum filter papers (Schleicher&-Schuell GF-52, 47 mm, nominal pore opening 0.7 *µ*m.) were dried in an oven at 105°C for 3 hours. When the papers were cooled at room temperature, their empty weights were weighed. Microalgae (100 mL) were filtered by a vacuum filtration system. The filter papers obtained after the filtration process were taken back to the oven at 105 °C and the drying process was carried out. After drying methods, the papers were taken into a desiccator. The dry matter (%) was calculated by measuring the papers that were cooled in the desiccator (AOAC, 1995). Kjeldahl method was preferred for crude protein analysis of microalgae. Microalgae samples were weighed 0.5 g–0.8 g and placed in Kjeldahl tubes (AOAC, 1995). Two pieces of Kjeldahl tablets and 20 mL sulphuric acid (H_2SO_4) were added to the microalgae samples placed in the tubes. The samples burned to 450°C for 120 minutes. The tubes were placed in the Kjeldahl device (Gerhardt VAPODEST®), and distillation and titration were performed. The amount of crude protein in the samples was calculated by determining the amount of 0.1N HCl consumed in the titration. Microalgae samples to be analyzed for crude lipid were weighed around 1 g and placed in the lipid extraction device. The glass VELPs (VELP® Scientifica), were previously dried in an oven at 105°C and kept in the desiccator. The samples were completed in the Soxhelet device for 60 minutes. After extraction, the glass VELPs were placed in an oven at 105 °C. The weights of the glass VELPs were weighed, and the percentage of crude lipid was calculated (Folch et al., 1957). Microalgae samples were placed in ceramic and burned in a muffle furnace at 550°C for 5-6 hours. The samples were taken into a desiccator to come to room temperature. The samples at room temperature were weighed. After weighing, the amount of ash was calculated (AOAC, 1995).

Fatty acid analysis

Fatty acid methyl esters were analyzed by GC (GC-2030; Shimadzu, Tokyo, Japan) in a Supercolvax-10 fused silica capillary column (constant pressure with 100KPa, length: 100 m; internal diameter: 0.25 mm; 0.20 i.d (Ref.: 24080-U) Supelco, Bellefonte, PA, USA) using H_2 as a carrier gas. Fatty acid methyl esters in algae biomass were gained by the transmethylation method with 1% sulfuric acid in methanol (Christie, 1982). The column temperature was 180 °C for the first 10 min, increasing to 260 °C at a rate of 2 °C min^{-1} and then held at 260 °C for 15 min. Then they were quantified by FID following the conditions described by Izquierdo et al. (1990) and identified by comparison with external standards well-characterized fish oils (EPA 28, Nippai, Ltd Tokyo, Japan).

Statistical analysis

Each sampling was conducted in triplicate and all data were treated with one-way analysis of variance (ANOVA) and the averages in the study were compared with the Duncan test ($p<0.05$) method in the SPPS program (SPSS for Windows 11.5; SPSS Inc., Chicago, IL, USA) and significance was adjusted at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance of microalgae

In this study, the growth performance of *Chlorella vulgaris*, *Pavlova lutheri*, *Euglena gracilis* and *Diacronema vlkanium* microalgae species were investigated. Growth performance was measured in two different volumes and time-lapse. The first measurement was between the initial and 15th days of culture and had a 250 mL culture volume while the second culture process was upscaled to 1000 mL culture volume with stable aeration between the 15th and 32nd days. The highest growth was determined in DV (*Diacronema vlkanium*) culture with 1.68×10^7 and 1.59×10^7 cells/mL density at 13th days and 30th days, respectively. Freshwater microalgae *Chlorella vulgaris* (CV) showed the highest growth rate on the 15th day of culture in 250 mL and cell density continuously increased until the end of the experiment with gentle aeration in 1000 mL erlenmayer. In terms of *Euglena gracilis* (EG) rapid growth was obtained between the 18th and 28th days in the presence of 1000 mL volume and regular aeration. The cell density is higher from the 20th and 28th days (3.50×10^6) compared to the culture of between the 2nd and 17th days (9.50×10^6). *Pavlova lutheri* (PL) maximum cell density was recorded at (7.77×10^6) at 15 days in and (8.50×10^6) at 32th days of culture, respectively. Microalgae growth performances are shown the Figure 2.

Proximate composition of microalgal biomass

In this study, the nutritional compositions were examined and it was reported that the highest crude protein content was found in *Chlorella vulgaris* (50.05±0.01%) and *Euglena gracilis* (42.15±0.52%) had the second highest level ($p<0.05$). However, the lowest crude protein (38.4±0.55%) was detected in marine microalgae *Diacronema vlkanium* ($p<0.05$). In terms of crude lipid content, the highest crude lipid (19.96±0.97 %) was found in marine microalgae *Pavlova lutheri* species whereas the lowest value was found in freshwater microalgae *Chlorella vulgaris* (11.2±0.02 %) ($p<0.05$). *Pavlova lutheri* had the highest dry matter (6.21±0.33%) content among groups ($p<0.05$). The table below shows the nutritional content of microalgae (Table 1).

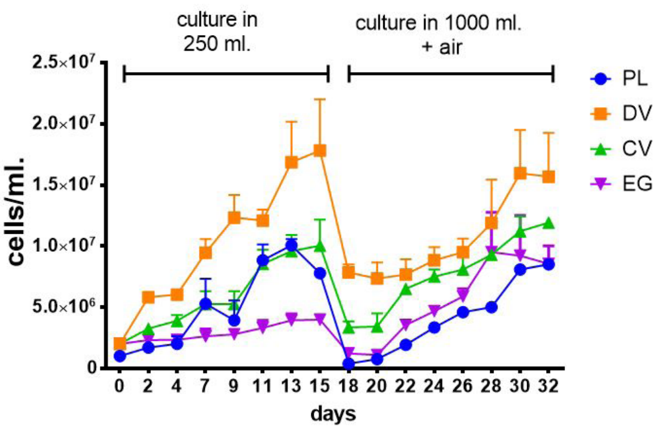

Figure 2. Growth performance of microalgae species; PL (*Pavlova lutheri*), DV (*Diacronema vlkanium*), CV (*Chlorella vulgaris*), and EG (*Euglena gracilis*).

Table 1. Proximate composition of microalgal biomass.

Proximate Analysis (%)	*Chlorella vulgaris*	*Euglena gracilis*	*Pavlova lutheri*	*Diacronema vlkanium*
Crude Protein	50.05±0.01[a]	42.15±0.52[b]	39.02±0.32[c]	38.4±0.55[c]
Crude Lipid	11.2±0.02[c]	15.35±0.31[b]	19.96±0.97[a]	18.01±0.99[a]
Crude Ash	7.2±0.00[c]	5.01±0.22[d]	10.01±0.88[b]	18.45±0.83[a]
Dry Matter	3.00±0.01[c]	2.44±0.00[d]	6.21±0.33[a]	5.89±0.96[b]

Dissimilar lettering show significant differences among groups (*p<0.05; Duncan's multiple range test).

Fatty acid composition of microalgae biomass

Microalgae is an important source of essential fatty acids such as EPA (C20:5n-3), DHA (C22:6n-3), and ARA (C20:4n-6) for aquaculture. *Euglena gracilis* had the highest EPA level (0.32±0.01%) among groups (p<0.05). The highest DHA level (1.79±0.02%) was found in *Euglena gracilis* biomass (p<0.05). *Euglena gracilis* and *Pavlova lutheri* had the highest ARA levels (3.32±0.05% and 3.16±0.00%) (p<0.05). Oleic acid (C18:1n-9) content highest values had *Chlorella vulgaris* and *Diacronema vlkanium* and the lowest value was *Euglena gracilis.* Freshwater microalgae had the highest (9.24±0.14% and 8.27±0.06%) linoleic acid (C18:2n-6) content (p<0.05). ALA (α-linolenic acid) (C18:3n-3) was found in *Euglena gracilis, Pavlova lutheri* and *Diacronema vlkanium* microalgae species with a level of 14.98±0.10%, 6.62±0.14% and 0.21±0.00%, respectively. γ-linolenic acid (C18:3n-6) was only found in *Pavlova lutheri.* The highest ∑ n-3 (24.87±0.03), ∑ n-6 (17.28±0.56) fatty acid contents were found in *Euglena gracilis.* Additionally, the highest ∑ n-3 HUFA (8.47±0.15%) and ∑ PUFA contents (39.58±1.33%) were found in *Euglena gracilis.* The lowest value (0.30±0.02%) was found in *Chlorella vulgaris* (p<0.05).

Microalgae are rich in lipids (Fields et al., 2014), carbohydrates (Chen et al., 2013), proteins (Becker, 2007), pigments (Begum et al., 2016), and fatty acids such as; PUFA, EPA and ARA (Eryalçın et al., 2013; Eryalçın et al., 2015) and therefore, they are very important future food supply not only for aquaculture purpose but also direct utilization of their biomass for human and animal diets. From this respect, recent studies are focused on the utilization of microalgae in both live prey feeding and formulated diets in marine fish. The nutritional value and growth performance of microalgae are also essential for biomass production. In microalgae culture, growth (doubling time), fatty acid content, and nutritional values are directly affected by the cultivation method. Moreover, the ingredients of the culture medium and stress conditions also affect the growth and proximate composition of the microalgae. For instance, *Scenedesmus sp.* can accumulate high levels of lipids under stress conditions (Khatoon et al., 2019). The other halophilic microalgae *Dunaliella salina* can contain a high amount of pigments under high salinity conditions (de Souza Celente et al., 2022), and freshwater microalgae *Chlorella vulgaris* may have high protein under low salinity conditions (Liu et al., 2008). In this study, the growth performance, proximate, and fatty acid composition of four microalgae cultured under constant laboratory conditions were investigated for potential aquaculture purposes such as live prey feeding or microalgae biomass.

Jeong et al. (2016) reported that the highest growth performance in *E. gracilis* was obtained by mixotrophic cultivation compared to phototrophic and heterotrophic cultivation methods with values of 2.48×10^6, 0.61×10^6 and 0.49×10^6 (cells/mL), respectively. A similar study was conducted by Gu et al. (2022) that calculated the growth performance of *E.gracilis* at autotrophic and mixotrophic culture methods and they reported autotrophic culture had a higher growth result (0.6×10^6 cell/mL) at 12 days of culture. In our study, *E.gracilis* was cultivated in a phototrophic way and found higher growth than other studies was obtained at a larger volume (1000 mL) with 9.2×10^6 (cells/mL), and lower growth was detected 3.97×10^6 (cells/mL) cell density in smaller culture (250 mL). In terms of fatty acid results, the highest levels of ALA, and EPA (14.92±0.10% and 4.79±0.08%) were obtained in phototrophic cultivation whereas the highest ARA, and DHA levels (3.39±0.03%, 1.74±0.02%) were found in mixotrophic cultivation method (Jeong et al., 2016). In our study, the highest levels of ALA, ARA, and DHA (14.98±0.10%, 3.32±0.05% and 1.79±0.02%) were found in *E.gracilis* biomass among microalgae whereas the EPA level (4.56±0.03%) was found the lowest in other microalgae species. This result could be related to different cultivation methods of the *Euglena gracilis*. Similar to Jeong et al. (2016) results, our study also showed that the phototrophic cultivation method enhanced fatty acid contents of *E. gracilis. Chlorella vulgaris* has a high potential for biomass production in both indoor and outdoor culture systems. It has been evaluated as feed ingredients in aquaculture (Ahmad et al., 2020). At laboratory scale production, we obtained the highest cell densitiy at 1.05×10^7 (cells/mL)

Table 2. Fatty acid composition of microalgae species.

Fatty Acid Compositions (% total fatty acid)	***Euglena gracilis***	***Pavlova lutheri***	***Diacronema vlkanium***	***Chlorella vulgaris***
C8:0	-	-	0.19±0.01	-
C10:0	-	0.06±0.00[b]	0.11±0.01[a]	0.03±0.00[c]
C11:0	-	0.62±0.02	-	-
C12:0	-	0.86±0.00[a]	0.44±0.03[b]	0.16±0.00[c]
C14:0	12.30±0.15[b]	1.01±0.01[c]	3.69±0.07[a]	0.60±0.02[c]
C14:1	-	-	-	0.11±0.00
C15:0	2.24±0.04[a]	0.14±0.01[c]	0.39±0.03[b]	0.26±0.01[b]
Iso16:0	5.81±0.03	-	-	-
C16:0	27.25±0.23[b]	24.50±0.09[b]	33.59±0.27[a]	18.80±0.21[c]
C16:1n-5	3.08±0.01	-	-	-
C16:2n-4	0.46±0.02	-	-	-
C16:1	-	0.57±0.03[b]	26.33±0.08[a]	28.76±0.18[a]
C17:0	0.90±0.01[a]	n.d.	0.35±0.03[b]	0.23±0.02[b]
C16:3n-4	1.26±0.02	-	-	-
C18:0	2.42±0.01[b]	1.14±0.01[b]	1.86±0.02[b]	14.38±0.05[a]
C18:1n-9	1.32±0.01[d]	15.94±0.02[c]	22.24±0.15[b]	28.21±0.13[a]
C18:2n-6	8.27±0.06[b]	9.24±0.14[a]	3.18±0.03[d]	7.46±0.01[b]
C18:2n-4	0.28±0.00	-	-	-
C18:3n-3	14.98±0.10[a]	6.62±0.14[b]	0.21±0.00[c]	n.d
C18:3n-6	-	1.05±0.01	-	-
C20:0	0.16±0.02[a]	-	-	0.14±0.01[a]
C20:1	-	0.70±0.01[a]	-	0.09±0.01[b]
C20:2	-	1.12±0.01	-	-
C20:2n-6	2.57±0.13	-	-	-
C20:3n-3	1.42±0.05[a]	-	-	0.24±0.00[b]
C20:3n-6	0.65±0.02	-	-	-
C20:4n-6	3.32±0.05[a]	3.16±0.00[a]	0.08±0.00[b]	-
C20:4n-3	1.72±0.02	-	-	-
C20:5n-3	4.56±0.03[b]	6.84±0.03[a]	2.20±0.03[c]	0.06±0.01[d]
C22:0	-	-	-	0.06±0.01
C22:4n-6	0.28±0.05	-	-	-
C22:5n-6	2.19±0.01	-	--	-
C22:4n-3	0.08±0.01	-	-	-
C22:5n-3	0.32±0.01	-	-	-
C22:6n-3	1.79±0.02[a]	-	-	0.09±0.02[b]
C24:0	-	0.08±0.00[b]	-	0.16±0.01[a]
Σ Monounsaturated	4.40±0.05[d]	17.21±0.04[c]	22.24±0.15[b]	29.0±0.16[a]
Σ Saturated	51.08±0.23[a]	28.61±0.13[d]	40.60±0.25[b]	34.8±0.18[c]
Σ n-3	24.87±0.03[a]	6.84±0.03[b]	2.40±0.03[c]	0.3±0.02[d]
Σ n-6	17.28±0.56[a]	13.45±0.13[b]	0.08±0.00[d]	7.5±0.01[c]
Σ n-9	1.32±0.22[d]	15.94±0.02[c]	22.24±0.15[b]	28.2±0.13[a]
Σ n-3 HUFAs	8.47±0.15[a]	6.84±0.03[b]	2.40±0.03[c]	0.30±0.02[d]
EPA/ARA	1.37±0.02	-	-	-
DHA/EPA	0.39±0.01	-	-	-
DHA/ARA	0.54±0.04	-	-	-
n-3/n-6	1.44±0.02[b]	0.51±0.01[b]	30.00±0.38[a]	0.04±0.02[c]
Σ PUFA	39.58±1.33[a]	26.90±0.03[b]	27.90±0.21[b]	7.61±0.04[c]

Dissimilar lettering shows significant differences among groups (*p<0.05; Duncan's multiple range test).

in 1000 mL volume at phototrophic culture. Taş and Dalkıran (2022) reported that the *C. vulgaris* initial cell densitiy was 1.1×10^6 (cells/mL) and the highest cell density obtained was 2.4×10^7 (cells/mL) on the 3rd day of mixotrophic culture. This higher algal productivity might be related to the nutrient composition of cultures medium by affecting the metabolism of microalgae cells (Fields et al., 2014). Light, temperature, and cultivation methods are important factors in microalgal growth and proximate composition. In our study, all parameters were constant therefore we assumed that growth and nutrient compositions were positively affected by culture mediums even when we used phototrophic culture methods.

Total lipid and protein accumulation should be higher in microalgae cells in order to be evaluated as feed ingredients. *E. gracilis* has distinctive features in the phototrophic cultivation method such as high protein content and high digestibility (Nwoye et al., 2017). In our study, the crude protein content of *Euglena gracilis* was higher than the marine microalgae species. On the other hand, the highest crude lipids were found in marine microalgae both *Diacronema vlkanium* and *Pavlova lutheri*. Yeh et al. (2010) found the crude protein and lipid contents of *C.vulgaris* as 25-30% and 30-40%, respectively. In our study, crude protein (50.05%) was found to be higher comparied to Yeh et al. (2010), moreover, the crude lipid (11.2%) value was lower. This result could be related to cultured microalgae in photobioreactor culture. Moreover, salinity highly affects of fatty acid contents of microalgae. Teh et al. (2021) investigated the fatty acid content of *C. vulgaris* at different salinity levels and oleic acid (C18:1n-9), linoleic acid (C18:2n-6), and α-linolenic acid (C18:3n-3) levels were found as 24.6%, 15% and 4.7%, respectively. In our study, we had higher oleic acid (C18:1n-9) (28.21%) and lower linoleic acid (C18:2n-6) (7.46%) levels compared to Teh et al. (2021). Marine haptophyte species *Pavlova lutheri* is known rich in protein and lipid content due to their large cell and ability to accumulate nutrients from culture water. *Pavlova lutheri* is known as rich in protein content among microalgae species (Shah et al., 2014). In our study, the crude protein content (39.02±0.32%) was detected highest value among four microalgae species. The other nutrients also showed good levels of crude lipid, crude ash, and dry matter contents at a level of 19.96±0.9%, 10.01±0.88%, and 6.21±0.33%, respectively.

Fradique et al. (2013) reported crude protein (38.4±0.2%), crude lipid (17.9±0.5%), crude ash (18.04±0.8%), and dry matter content (91.03±0.01%) determined in ‰25 salinity culture conditions of *Diacronema vlkanium*. In our study, salinity was adjusted at ‰30 – ‰32 salinity, the contents of crude protein, lipid, ash, and dry matter were found as 38.4±0.55%, 18.01±0.99%, 18.45±0.83%, 5.89±0.96%, respectively. In another study, Cañavate and Fernández-Díaz (2022) showed lipid and fatty acid composition of *D.vlkanium* at different salinity levels. According to this study, EPA and DHA levels were found 7.6% and 6.6% of total fatty acids between ‰20 - ‰35 different salinity ranges in *D. vlkanium* production. In our study, essential fatty acids showed moderate levels of EPA and DHA (0.06±0.01% and 0.09±0.02%) at similar salinity levels. This result could be related to the fatty acid elongation of microalgae as long as salinity increases (Cañavate and Fernández-Díaz., 2022). We assume that EPA and DHA levels were linearly correlated with a high salinity in *D.vlkanium* species. In terms of marine haptophyte *Pavlova lutheri* has essential fatty acids such as EPA and ARA of total fatty acids. We obtained high accumulation of EPA (6.84%) and ARA (3.16%) levels in this haptophyte algae.

As a result, *D. vlkanium* can be cultured with the highest growth performance under phototrophic cultivation when compared to other microalgae species that were examined. However, all microalgae enhanced cell density after the 18th day of the experiment due to gentle aeration and flow current of culture water. The aeration positively effects the microalgal cell density and growth performance due to increases in the amount of CO_2 and nutrient content in the photrophic culture conditions (Mohsenpour and Willoughby, 2016). *Euglena gracilis* and *Chlorella vulgaris* are productive species together with high protein and biomass contents. Dry matter is important when powder product is concerned with microalgae. The highest dry matter content was found in *Pavlova lutheri*. From this point, *P.lutheri* is a suitable species for the production of biomass and turn into dry material which features of highest dry matter content. *Euglena gracilis* have high content of ALA, ARA, EPA, and DHA which are important for fish feed raw materials (Wang et al., 2018). Microalgae fatty acid contents depend on the aeration, amount of CO_2, light intensity, temperature, and culture medium (Schwarzhans et al., 2015, Guedes et al., 2010, Go et al.,2012). In our study, the highest ALA, ARA, and DHA fatty acids contents were found in *E. gracilis*. This result could be related to, the cultivation of microalgae by phototrophic methods. However, the highest EPA content was found in *Pavlova lutheri*. EPA is highly important for fish feeding and larval development. EPA and DHA fatty acids are very difficult to synthesize from fish (Guedes et al., 2010). That's the reason why the aquaculture industry has to use rich EPA and DHA contents from microalgae species.

CONCLUSION

In conclusion, within this study, we applied the same microalgae culture procedure at commercial hatcheries in our laboratory where microalgae culture start with small vessels and then continuously inoculated a large volume with gentle aeration. The purpose of this work was to investigate both the growth of algae during 32 days of culture (250 mL and 1000 mL glass flasks) and nutritional value and fatty acid composition at the end of the 32nd day of culture just before they were inoculated in 30 L plastic bags. The data obtained from our laboratory is valuable for both commercial hatcheries where those microalgae are utilized. To sum up, the success of microalgal up-scale culture in both freshwater and marine microalgae species are strongly related to inoculation time and volume. As a result, all microalgae have a high potential for biomass gain in a very short time with good enough nutrients. Moreover, *Pavlova lutheri* and *Euglena gracilis* can supply promising levels of highly unsaturated essential fatty acids such as ARA, EPA, and DHA. Most importantly, we suggest based on obtained data these two microalgae have high potential for dry biomass production due to their high dry matter content. Therefore, those biomass have high potential to use feed ingredients in aquaculture and this can lead to a positive effect on sustainable production. Additionally, we conclude that laboratory-scale production of those four microalgae should be inoculated from a 250 mL culture flask to 1000 mL flasks at two weeks. Microalgae biomass production and its nutrient compositions are affected by culture systems like photobioreactors, volumes, and culture types such as phototrophic, myxotrophic, and heterotrophic culture. Further studies are needed for larger photobioreactor production and biomass investigations.

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