Journal of Spectroscopy and Molecular Sciences

Journal Homepage: https://dergipark.org.tr/tr/pub/jsms

Growth of microalgae (*Chlorella vulgaris*) in the presence of olive leaf extract

Şule Ören[1], Zeynep Yıldız[1], Merve Deniz Köse[1], Kübra Potuk[1], Oguz Bayraktar[2,*]

[1]Department of Chemical Engineering, Ege University, Izmir, Turkey
[2]Department of Bioengineering, Ege University, Izmir, Turkey

*Correspondence: oguzbayraktar70@gmail.com
ORCID Oguz Bayraktar: https://orcid.org/0000-0003-4210-2825

Abstract: Microalgae has been used for various applications in the literature. Microalgae can produce different biologically active metabolites due to their different morphological, physiological, and genetic traits. In this study, biotransformation of olive leaf extract by microalgae under biotic conditions was investigated. The results showed that incorporating the olive leaf extract into the growth medium changed the microalgae's specific growth rate and its total phenolic content. The effect of type of light (white and red light) on the specific growth rate was also investigated. The obtained data showed that light type directly changed the specific growth rate of the microalgae. With their growth under both white and red light, the olive leaf extract amount in the growth medium decreased while the medium's antioxidant capacity increased. This was attributed to the production of bioactive compounds due to the biotransformation of polyphenols by microalgae.

Keywords: Microalgae; Olive leaf extract; Phenolic compounds; Biotransformation.

1. Introduction

Microalgae have been used as an inexpensive source for valuable metabolites such as β-carotene, astaxanthin, and algal extracts in cosmeceuticals, nutraceuticals, and functional foods (Borowitzka, 2013; Khan et al., 2018). Microalgae can increase conventional food preparations' nutritional content and positively affect humans' and animals' health due to their original chemical composition. Comparison of the general compositions of familiar human food sources and different algae were presented in Table 1.

Tang and co-workers gathered a review of the potential utilization of bioproducts from microalgae. Microalgae-produced bioactive compounds include lipids, peptides, amino acids, antioxidants, polysaccharides, and pigments (Tang et al., 2020).

Even though algae are very easy to cultivate, there are some limitations in microalgae production. Various environmental factors are known to affect microalgae growth. Light is one of the main parameters that affect algae's growth rate (Gatamaneni Loganathan et al., 2020).

Received 20 December 2020, Accepted 31 December 2020, Available online 31 December 2020.

Table 1. General composition of different human food sources and algae (% of dry matter) (Spolaore et al., 2006).

Commodity	Protein	Carbohydrate	Lipid
Meat	43	1	34
Milk	26	38	28
Rice	8	77	2
Chlorella Vulgaris	51–58	12–17	14–22
Dunaliella salina	57	32	6

Nzayisenga and co-workers studied the effects of light intensity on growth and lipid production in microalgae grown in wastewater. Their results showed that an increase in light intensities resulted in higher biomass for all microalgae species. Fourier-transform IR (FTIR) spectrometry analysis showed that the increment in fatty acid content was attributed to reductions in protein, but not carbohydrate, contents. When the fatty acid composition was assessed, increasing light intensity led to higher and lower contents of oleic (18:1) and linolenic (18:3) acids, respectively (Nzayisenga et al., 2020).

With the increase in cell density, light available for photosynthesis inside the culture vessel is decreased due to self-shading, scattering, and absorption by the cells. To overcome these problems, photobioreactors were proposed. In recent years, LEDs have gained popularity due to their compactness, longer lifetime, high electrical conversion efficiency, and, most importantly, low heat emission (Yuvraj et al., 2016). With photobioreactors, the microalgae can capture the light energy better. In our previous study, the design of photobioreactors for the production of *Chlorella sp.* was studied. The photobioreactor system was designed in consideration of good aeration, good mass transfer characteristics, proper lighting. As an artificial light source, led strips with equal lengths 120° were chosen, and a hexagonal plexiglass container was designed to achieve homogeneous light distribution. The designed photobioreactors were used due to these reasons (Köse et al., 2019).

Reymann and co-workers assessed the biotic and abiotic elimination processes of micropollutants by using microalgae. Results showed that present micropollutants stressed microalgae in the growth medium, limiting their ability to eliminate these pollutants. It was shown that stressed microalgae change their composition to cope with the stressful environment. This resulted in a change in the lipid content in the microalgae cell. Obtained data showed that the overall growth of this culture is comparable with the control group. The microalgae's ability to eliminate the micropollutants was not affected by the inhibition of the growth of the cells. The elimination of a substance during microalgae cultivation alone cannot conclude which process caused the elimination because the elimination process includes photolysis, sorption, and microalgae. However, for one micropollutant (Sulfamethoxazole-SMX) was not eliminated by photolysis or sorption due to this reason, its elimination can be attributed to the microalgae. After 172 h of cultivation, 43% of the SMX was eliminated (Reymann et al., 2020).

Traditionally, microalgae were used as a source for phenolic compounds, or they were used to separate and purify the phenolic compounds from wastewater systems. Recent applications of phenolic compounds as a carbon source for the biotechnological processes to obtain value-added products from a metabolically versatile microorganism had gained a lot of interest (Lindner & Pleissner, 2019).

In a review prepared by Hernández and co-workers, algal enzymes and their potential biotechnological uses were explained. In the paper, carbonic anhydrase, hydrogenase, lipoxygenase, nitrilase, nitrogenase, phosphatase, and thiolase were listed as algal enzymatic complexes (Hernández et al., 2017).

Greca and co-workers used 11 microalgae strains for the biotransformation of ethinylestradiol (EE). Their results showed that out of 11 microalgae strains, only 4 microalgae strains; *Selenastrum capricornutum, Scenedesmus quadricauda*, *Scenedesmus vacuolatus,* and *Ankistrodesmus braunii,* had biotransformed the substrate. When optimum algal density conditions were chosen, it was seen that EE was converted with 92% yield in ethinylestradiol glucoside by *S. capricornutum* (Della Greca et al., 2008).

Pinto et al., *Ankistrodesmus braunii* and *Scenedesmus quadricauda* were used to remove the olive oil mill wastewater phenolic compounds. Both light and dark growth conditions were used. HPLC results showed that catechol and hydroxytyrosol, which are the main phenolic component in the olive mill wastewater, were entirely removed by both strains. In addition to that, new peaks were observed in the HPLC chromatogram after the algal treatment, which was interpreted as biotransformation of the phenols present in the wastewater (Pinto et al., 2003).

El-Sheekh et al., green algae species were used for the biodegradation of phenolic and polycyclic aromatic compounds. The obtained data showed that 83% and 92% naphthalene was removed by the green algae *Chlorella sp.* and *Elakatothrix sp.*, respectively. These results suggested that the mechanism of physiological metabolism used to biotransform phenolic compounds strongly depends on the molecular structures of phenolic compounds and species used (El-Sheekh et al., 2012).

Olive leaves, which are agricultural waste, have great potential as a natural antioxidant. Many studies investigated the phenolic content in the literature and assessed the antioxidant and antimicrobial activities of both the individual and combined phenolics in olive leaf extract (Castro et al., 2020; Lee & Lee, 2010; Şahin et al., 2011; Somerville et al., 2019). In the literature, oleuropein was identified as the primary active compound in the olive leaf known for its health benefits. Oleuropein, a secoiridoid compound, is present in the whole *Olea europaea L.* olive tree and its derivatives (olive oil, olive mill wastewater, and pomace). It is the most abundant phenol and the primary bioactive compound in olive leaves. In Figure 1, the molecular structure of oleuropein is given.

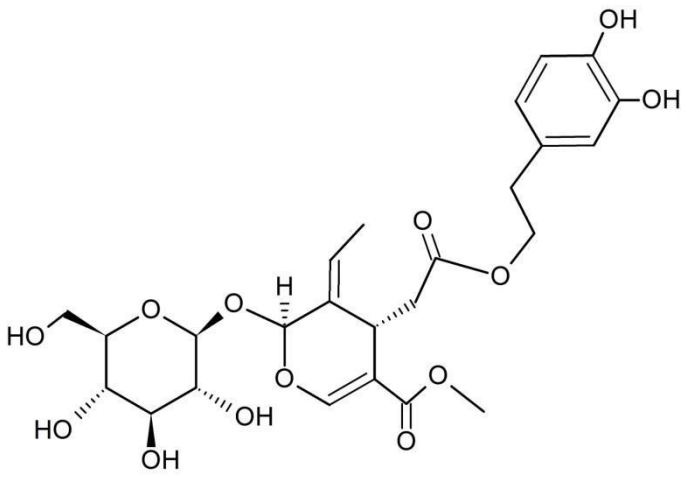

Figure 1. Molecular structure of oleuropein.

As stated before, the usage of microalgae for biotransformation has been recently increased. In our study, olive leaf extract (OLE) was used as a phenolic source in the growth medium to investigate phenolic compounds' biotransformation by microalgae (*Chlorella vulgaris*) under different light types. In the literature for the characterization of phenolic compounds HPLC, NMR, TLC and FTIR analysis were performed. In FTIR, spectra of the microalgae samples cultivated under different conditions and spectra of the extract obtained from algal biomass were studied. HPLC chromatograms were used for the determination of oleuropein amount in the growth medium.

2. Materials and methods

2.1. Materials

Olive leaves were collected from the Ege University campus (Turkey). Ethanol was used to extract olive leaves, and it was obtained from Merck (Germany). Folin-Ciocalteu was used to determine the total phenol content obtained from Sigma (USA). Sodium carbonate anhydrous (99.5%) was purchased from Carlo Erba (Spain). HPLC grade acetonitrile was purchased from Carlo Erba (Spain). Trolox (6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid), ABTS (2,'2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) reagents, and potassium persulfate ($K_2O_8H_8$) from Merck (Germany) was used for antioxidant analysis.

2.2. Growth medium and culture conditions for *Chlorella vulgaris*

Chlorella vulgaris was cultivated in Bold's basal medium (BBM) in two-column photobioreactors. In the previous study, designed photobioreactors were with led strips with 120° on each surface of the hexagonal plexiglass. Led strips were assembled with equal lengths on each surface. The light source's distance to the reactor surface was set as 2.5 cm from the edge to the led container, and the reactor was bought with a diameter of 15 cm (Köse et al., 2019). The designed photobioreactors are shown in Figure 2.

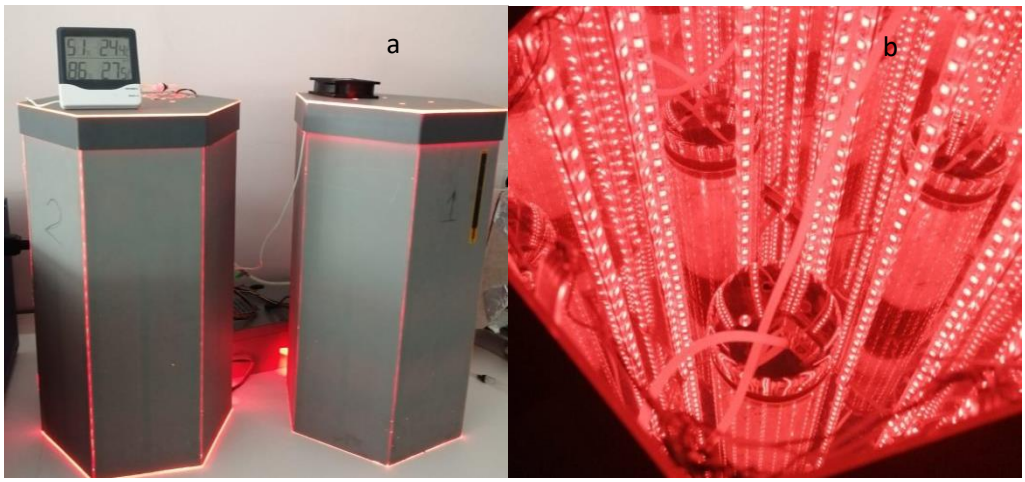

Figure 2. The designed photobioreactors a) outer view b) interior view under red light.

As reported in the literature, BBM components can be listed as $NaNO_3$, $MgSO_4.7H_2O$, NaCl, K_2HPO_4, KH_2PO_4, $CaCl_2.2H_2O$, H_3BO_3, EDTA-KOH solution, $FeSO_4.7H_2O$-der.H_2SO_4 solution, and trace metals solution. Trace element solution components are $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, MoO_3, $CuSO_4.5H_2O$, and $Co(NO_3)_2.6H_2O$. The pH of BBM was 6.7. The prepared mixture was prepared in 1 liter of deionized water. After the medium was prepared, it was sterilized in an autoclave at 124 °C for 20 minutes to prevent contamination and cool down.

The air pump (Atman Aquarium Air Pump, HP-8000) was used for CO_2 and O_2 supply with a 3 L/min aeration rate. Microalgae production was performed under room conditions (25 ºC temperature-1atm pressure).

2.3. Preparation of olive leaf extract (OLE)

Olive leaves are washed first and then dried and ground. Ground olive leaves were used in the extraction. Extraction was done with a 1:30 solid to liquid ratio in 70% aqueous ethanol solution for 24 hours. After 24 hours, filtration was done to remove insoluble parts in the extract. The remaining mixture was lyophilized after the excess alcohol in the mixture was evaporated on a rotary evaporator.

2.4. Determination of proper OLE amount in the growth medium

After the olive leaf extract was dried by lyophilization, it was used as a phenolic source in the growth medium with different concentrations. The effect of extract concentration on growth and antioxidant capacity were investigated. The amount of olive extract was determined by trial and error as 6, 3, 2.5, 2, 1.5 g per 1000 ml of BBM. First, 6 g of extract was added for 1000 ml. Saponin contained in olive leaf extract caused excessive foaming (Góral & Wojciechowski, 2020). As a result, it caused losses in the solution, and the experiment was stopped because the correct result could not be obtained. 6 g was determined as the upper limit. Therefore, the amount of extract decreased. For the new experiment, 3 g of extract was added to the 1000 ml solution. Growth was observed, but the targeted 8-day growth could not be achieved. As in the first attempt, foaming

occurred, and the water in it evaporated completely. As a result of these, 2.5 g and 1.5 g were decided for the experiments.

2.5. Effect of light type on the growth

After the optimum OLE concentrations (C_1 and C_2) were determined, the effect of light wavelength on the cell growth was investigated. The changed parameter was the color of light. For this purpose, the white and red light was used, and turbidity values were taken day by day in the UV spectrophotometer as 2 ml samples, and the measurements were read at 450 nm.

2.6. Calculation of specific growth rate

For the growth rate, dry weight measurements were taken. For dry weight, 2 mL samples were taken from the cultures. The filtering process was carried out using filter papers. The sample taken for dry weight was left in the oven at 60 °C. The dried filter papers were weighed with a balance on the next day, and the dry weight values per liter were calculated in grams.

Specific growth is the slope of the growth rate curve in the exponential phase (Metsoviti et al., 2019). The specific growth rate was determined from the obtained growth rate graph.

$$\mu(\boldsymbol{day^{-1}}) = \frac{\boldsymbol{ln}\,(\boldsymbol{x_2}) - \boldsymbol{ln}\,(\boldsymbol{x_1})}{\boldsymbol{t_2(day) - t_1(day)}} = \frac{\boldsymbol{ln}(\frac{\boldsymbol{x_1(mg)}}{\boldsymbol{x_2(mg)}})}{\boldsymbol{t_2(day) - t_1(day)}}$$

where μ is the growth rate (day), x_2, and x_1 (mg) dry weight of biomass at times t_2 and t_1, respectively.

2.7. Characterization and determination of the biomass composition

Dried algal biomasses were characterized by Fourier Transform Infrared (FTIR) Spectroscopy. The samples' spectra were recorded in 650 to 4000 cm^{-1} using Perkin Elmer Spectrum 100 FTIR spectrometer. 8±0.2 mg samples were analyzed with ATR mode.

Biomass composition was determined according to the literature. The method developed by Pistorius and co-workers was used in the study for the determination of lipid and protein content of the biomass (Pistorius et al., 2009). In their study, the amide II band was used for the quantitative estimation of protein content. The two-point baseline correction followed by integration between 1590 and 1477 $cm^{-1,}$ the calibration curve for the protein amount was given as $y = -0.27 + 12.72x$ (correlation coefficient r^2=0.994). Quantitative evaluation of lipid content was determined from the band around 2850 cm^{-1} with the calibration curve $y = -2.30 + 78.96x$ (correlation coefficient r^2=0.982) where x represents the sample's content (in mg unit) and y the integrated area under the vibrational band.

2.8. Preparation of extracts from algal biomass

For the extraction of algal biomass, studies from the literature were used. Dried microalgae biomass (12.5 mg) was extracted with 1 ml of acetone for 24h at room temperature under dark conditions (Abdel-Karim et al., 2019; Jerez-Martel et al., 2017). After centrifugation at 3000 rpm for 10 min (Centurion, Scientific Limited), the supernatant was collected and filtered with a syringe filter and analyzed with HPLC UltiMate 3000, Thermo Scientific, USA.

2.9. Determination of the oleuropein in the prepared extracts

The chromatographic analysis was performed on Thermo Scientific Acclaim™ 120 C18 Columns (5µm, 4.6 x 250mm). The mobile phase was prepared with HPLC grade acetonitrile and acetic acid (97.5:2.5). UV detection was used at 280 nm, isocratic elution was used at a flow rate of 1.0 mL/min, and the injection volume was set to 20 mL. The stock standard solution of oleuropein with a concentration of 1.35 mg/ml was prepared with acetonitrile. Serial dilutions were done with acetonitrile, and the calibration curve was prepared at 280 nm for standard oleuropein. The analyses were carried out with UltiMate 3000, Thermo Scientific, USA.

2.10. Determination of antioxidant capacity

As stated in the literature, 7 mM aqueous ABTS solution was mixed with 2.45 mM potassium persulfate ($K_2S_2O_8$) solution to form $ABTS^+$ and left in the dark for 16 hours at room temperature (Altıok et al., 2008). The obtained $ABTS^+$ solution was diluted with methanol to obtain an absorbance value of 0.7 (±0.02) at 734 nm.

The samples from the algae cultures were diluted 1/10. 10 µl sample was put in the bath, then 200 µl ABTS was added. All the measurements were repeated three times. After waiting for half an hour, the absorbance values were read. Absorbance results were first converted to percent inhibition value, and then the antioxidant activity of the samples was determined by a calibration curve with Trolox equivalent (Altıok et al., 2008; Rajurkar et al., 2011).

2.11. Determination of total phenolic content

Total phenol content analysis of the samples was done by the Folin-Ciocalteu method using Gallic acid as a standard. Folin-Ciocalteu reagent was diluted with a 1:10 ratio. The Na_2CO_3 solution was prepared as 7.5 % (m/v). The samples from the algae cultures were diluted 10 fold. 20 µl sample, 100 µl Folin reagent, and after 2.5 minutes, 80 µl Na2CO3 were added. All the measurements were replicated three times, and their arithmetic mean was taken. Prepared samples were kept in the dark for an hour.

3. Results and discussion

3.1. Growth of microalgae

3.1.1. Determination of proper olive leaf extract (OLE) concentration for the growth of microalgae

As explained in the materials and method section considering the amount of foaming problem during the growth of microalgae, 1.5 mg/ml (C1) concentration of OLE was found to be suitable for completing the growth phase without severe foaming problem. In order to determine the effect of the extract concentration, an experiment was carried out with a higher OLE concentration (C2 2.5 mg/ml) as well. In Table 2, performed experiments, and their conditions are summarized.

Table 2. Experiments for microalgae growth in a medium with different OLE content and light types.

Type of light	Exp. code	OLE concentration in microalgae growth medium (mg/ml)	Specific growth rate (day^{-1})
White	C_{0W}	0 (Control)	0.17
	C_{1W}	1.5	0.34
	C_{2W}	2.5	0.38
Red	C_{0R}	0 (Control)	0.43
	C_{1R}	1.5	0.48

The growth rate changes of microalgae are given in Figure 3.

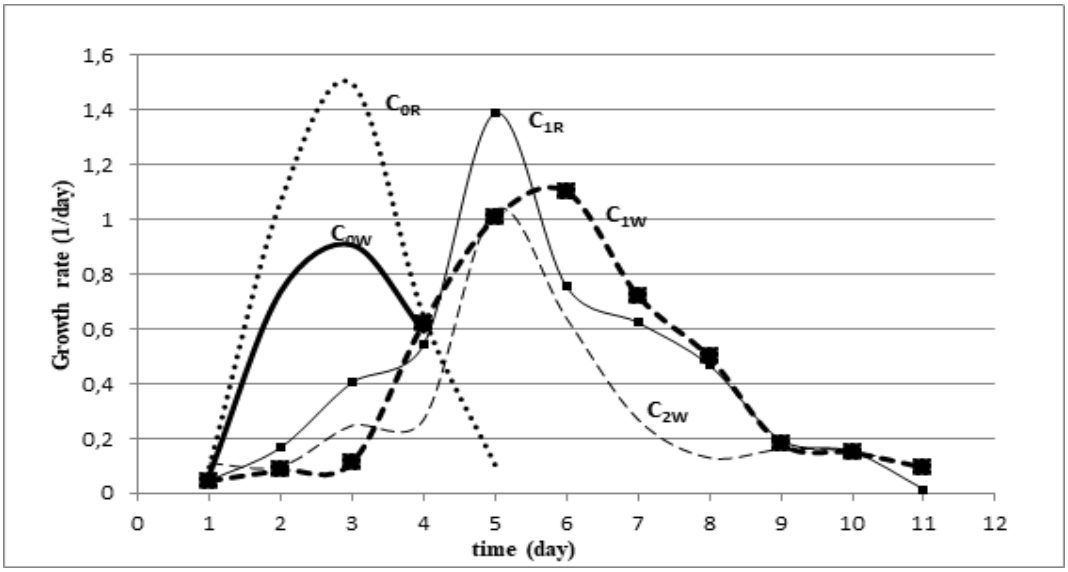

Figure 3. Growth rates of microalgae grown a) without OLE (C_{0W}) under white light b) without OLE (C_{0R}) under red light c with OLE (C_{1R}) under red light d) with OLE (C_{1W}) under white light e) with OLE (C_{2W}) under white light.

As seen from Figure 3, the highest growth rate was observed for microalgae cultivated under red light without OLE. The second-highest growth rate was achieved for microalgae cultivated under red light with an OLE concentration of 1.5 mg/ml (C_1). Under the red light addition of OLE into the growth, the medium decreased the growth rate. However, for the white light conditions, the addition of OLE into the growth medium increased the growth rate. However, the further increase of the OLE concentration to 2.5 mg/ml (C_2) had a negative effect on the growth rate.

While calculating the specific growth rate, Figure 3 was used. The calculated specific growth rates are tabulated in Table 2.

Table 2 shows that the higher specific growth rate values were achieved for the microalgae growth under the red light conditions. Two parameters were examined with the addition of OLE. The first was the effect of the OLE concentration, and the second was the effect of light type at constant OLE concentration. According to the values of 1.5 (C_1) and 2.5 mg/ml (C_2) under white light, it can be said that when the amount of OLE was increased, the specific growth rate increased as well. According to the light type, growth under red light resulted in relatively higher specific growth rates with the addition of OLE into the growth medium.

3.2. The effect of light type on the growth rate

In the study, five different experiments were carried out, which are listed in Table 2. As a result of these experiments, turbidity changes indicating the growth of microalgae over time are given in Figure 4.

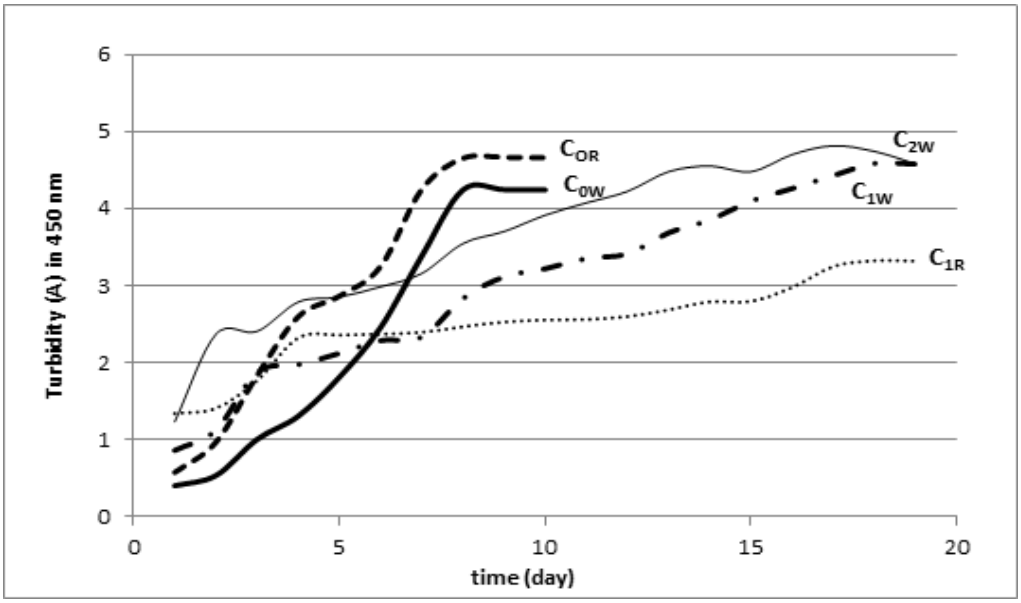

Figure 4. Turbidity changes during the growth of microalgae. Microalgae growth; C_{0W} algae without OLE under white light C_{0R} algae without OLE under red light C_{1R} algae with OLE under red light C_{1W} algae with OLE under white light C_{2W} algae with OLE under white light.

When the absorbance values measured at 450 nm with a UV spectrophotometer were examined, it was observed that under white light, the growth without OLE ended earlier than the growth with OLE concentrations of C_1 and C_2 as 1.5 and 2.5 mg/ml, respectively. The OLE-free microalgae were kept growing up to 9 days (C_{0W}). However, this growth period extended to 18 days,

with OLE concentrations of 1.5 and 2.5 mg/ml (C_{1W}, C_{2W}). When the effect of the light type on the growth of microalgae without OLE was compared, it was observed that the microalgae growth under red light (C_{1R}) was higher than the growth under white light (C_{0W}). When the growth with OLE concentration of 1.5 mg/ml under both lights was compared, it was observed that growth under red light (C_{1R}) was less than growth under white light (C_{1W}).

The duration seen on the 7th day in the (C_{0W}, C_{0R}) was observed in both control groups (without OLE), the difference between slopes caused by the oxygen limitation. Oxygen limitation has not occurred in OLE added sets, as a result, the microalgae growth extended to 19 days. The oxygen limitation affects the system more when the microalgae quickly go through the death phase (Sarian, Rahman, Schepers, & van der Maarel, 2016).

Dry weight changes during microalgae growth over time are given in Figure 5.

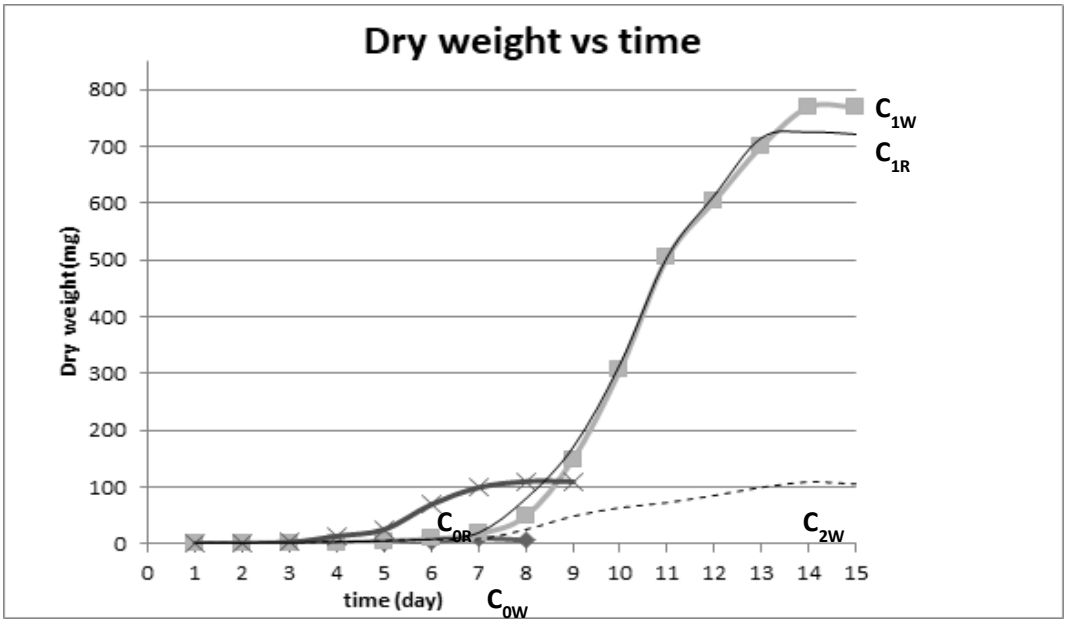

Figure 5. Dry weight changes during the growth of microalgae. Microalgae growth; C_{0W} algae without OLE under white light C_{0R} algae without OLE under red light C_{1R} algae with OLE under red light C_{1W} algae with OLE under white light C_{2W} algae with OLE under white light.

As seen in Figure C_{0R}, a higher dry weight of microalgae biomass was achieved under red light without OLE. Yan and co-workers studied the effect of various light wavelengths on algae's growth and nutrient removal efficiency. They concluded this red light was the optimum light wavelength for the best growth rates in that study. The red light wavelength resulted in the highest microalgae dry weight and nutrient removal efficiency (Yan et al., 2013). The addition of OLE at a concentration of 1.5 mg/ml had a positive effect on the microalgae's dry weight. The highest biomass dry weight values were achieved under both white and red lights (Fig.C_{1R}, C_{1W}). However, a decrease was observed in the dry weight of microalgae (Fig. C_{2W}) under white light when OLE concentration was increased to 2.5 mg/ml. With OLE addition to the growth medium, the color of the growth medium changed from green to brownish color. Matthijs et al. made an explanation for the color change as green pigment chlorophyll in microalgae effectively absorbs red light wavelength. So with the addition of OLE to the growth medium changed the absorption of the light (Matthijs et al., 1996).

Microalgae biomass concentration changes of the performed growth experiments are given in Figure 6.

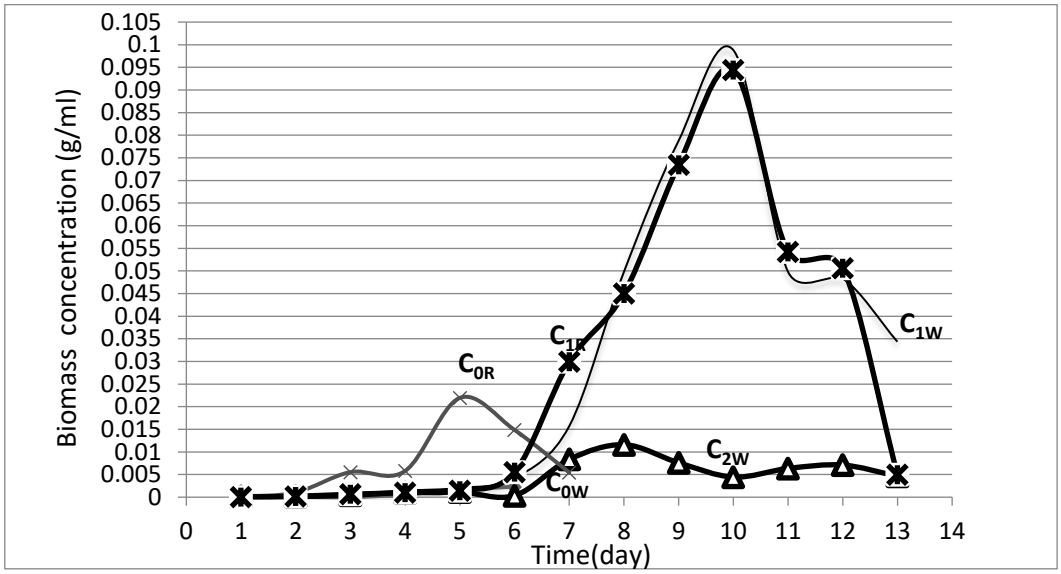

Figure 6. Biomass concentration changes during microalgae growth; C_{0W} algae without OLE under white light C_{0R} algae without OLE under red light C_{1R} algae with OLE under red light C_{1W} algae with OLE under white light C_{2W} algae with OLE under white light.

As seen from Figures C_{1R} and C_{1W}, higher biomass concentrations were achieved with an OLE concentration of 1.5 mg/ml for the microalgae growth under both white and red lights. When OLE concentration in the growth medium increased to a concentration of 2.5 mg/ml, a significant decrease in the biomass concentration for the growth under white light was observed in Figure C_{2W}.

3.3. Characterization of microalgae biomass and their extracts

To investigate the effect of OLE and light type, samples were analyzed with FTIR. In Figure 7, the FTIR spectra of the microalgae samples cultivated under different conditions are given.

As seen from Figure 7, all samples showed the same characteristic peaks. Each peak was assigned specific functional groups. In the spectra, the protein was characterized by two peaks at 1643 and 1543 cm^{-1}. In the literature, these bands identified as C=O stretching vibration and a combination of N-H bending and C-N stretching vibrations in amide complexes, respectively. Lipid in the spectra was characterized with bands the C-H at 2928 cm^{-1} and the C=O mode of the side chain from ester carbonyl group at 1737 cm^{-1}, carbohydrate absorption bands due to C-O-C of polysaccharides at 1149, 1082, and 1036 cm^{-1} (Dilek (Yalcin) et al., 2012).

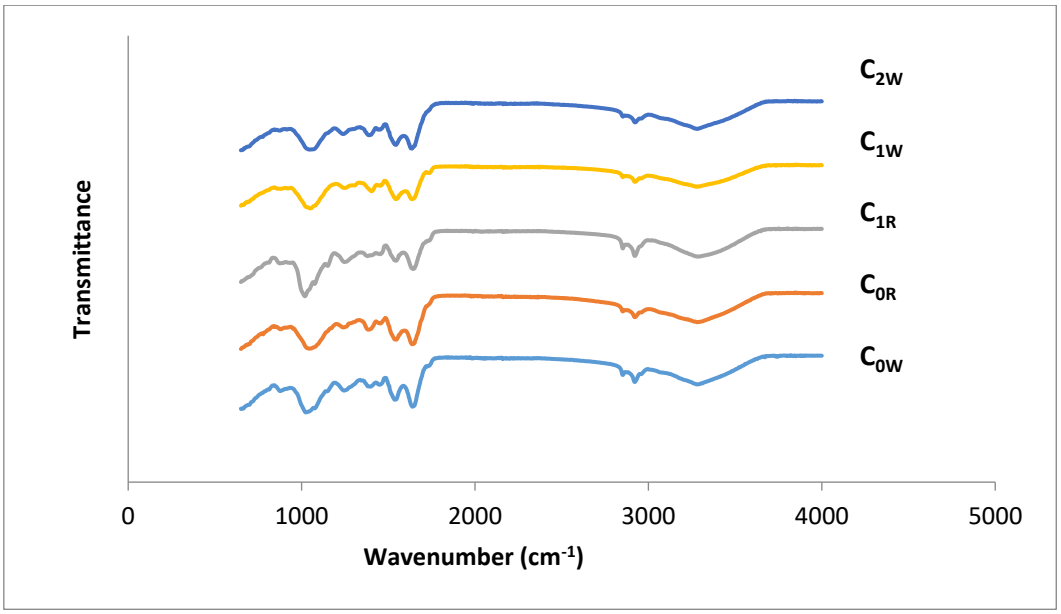

Figure 7. FTIR spectra of microalgae biomass grown; C_{0W} algae without OLE under white light C_{0R} algae without OLE under red light C_{1R} algae with OLE under red light C_{1W} algae with OLE under white light C_{2W} algae with OLE under white light.

The FTIR spectra of the extract obtained from algal biomass are given in Figure 8.

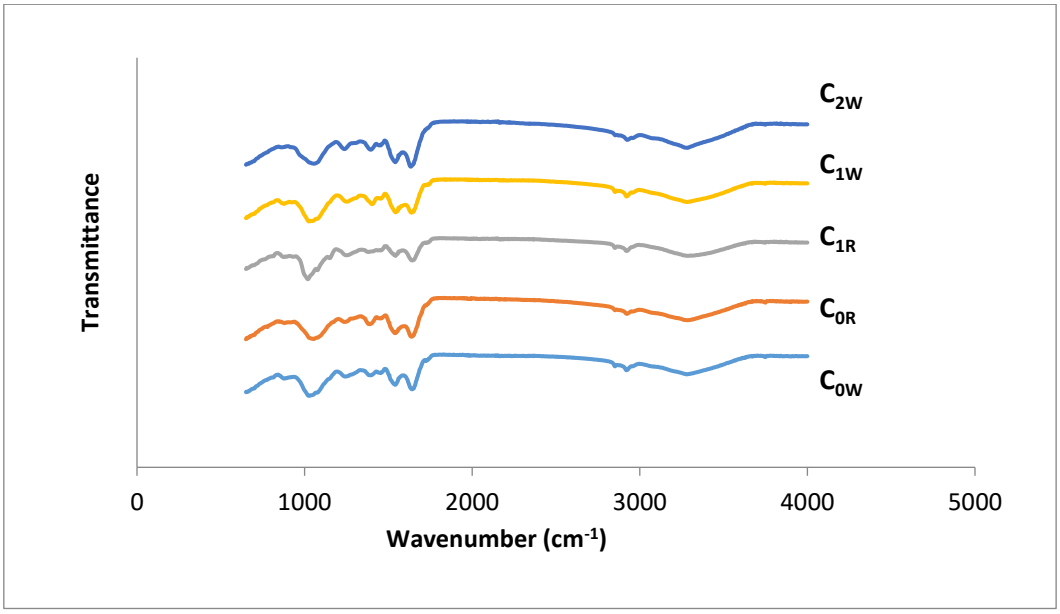

Figure 8. FTIR spectra of extracts obtained from microalgae biomass C_{0W} algae without OLE under white light C_{0R} algae without OLE under red light C_{1R} algae with OLE under red light C_{1W} algae with OLE under white light C_{2W} algae with OLE under white light.

As seen from Figure 8, FTIR spectra of the extracts obtained from microalgae biomass showed the characteristic peaks similar to the biomass. However, slight decreases were observed in peak intensities (Dilek (Yalcin) et al., 2012).

3.4. Protein and lipid amount of the microalgae biomass

The calculated protein and lipid contents of the algal biomass are given in Table 3.

As seen from Table 3, for biotic conditions under the white light addition of OLE to the growth medium caused an increase in the lipid content of biomass while causing protein content to decrease. Similarly, microalgae cultivation under red light with the addition of OLE to the growth medium decreased the biomass's protein content while increased the lipid content (Ferro, Gojkovic, Gorzsás, & Funk, 2019).

Table 3. Protein and lipid contents of the microalgae biomass.

Light type	**Exp. code**	**Protein (mg) (Amide II band)**	**Lipid (mg) (CH_2 stretching)**
White	C_{0W}	0.919	0.212
	C_{1W}	0.840	0.227
	C_{2W}	0.829	0.263
Red	C_{0R}	0.935	0.267
	C_{1R}	0.612	0.359

3.5. Results of HPLC analysis

According to the calibration curve prepared with standard oleuropein, the oleuropein amount in the olive leaf extract used in the experiments determined as 22 %. HPLC chromatograms of samples from microalgae growth medium initially for growth under white and red are given in Figure 9 a,b. HPLC chromatograms of samples from microalgae growth medium after growth completed under white and red are given in Figure 9 c,d, respectively. For both under white and red light, oleuropein amount in the growth medium decreased significantly. These results indicated that oleuropein could be consumed during the growth of microalgae. After carefully investigating the chromatograms and the concentrations of oleuropein, it was observed that the utilization of oleuropein during the microalgae growth under white light was slightly higher than that for microalgae growth under red light.

It can be speculated that polyphenols like oleuropein can be hydrolyzed, and the glucose moieties released from its structure after hydrolysis can be used for the growth of microalgae.

However, after increasing OLE concentration, in our case to 2.5 mg/ml growth of microalgae affected negatively as determined in our specific growth rate results.

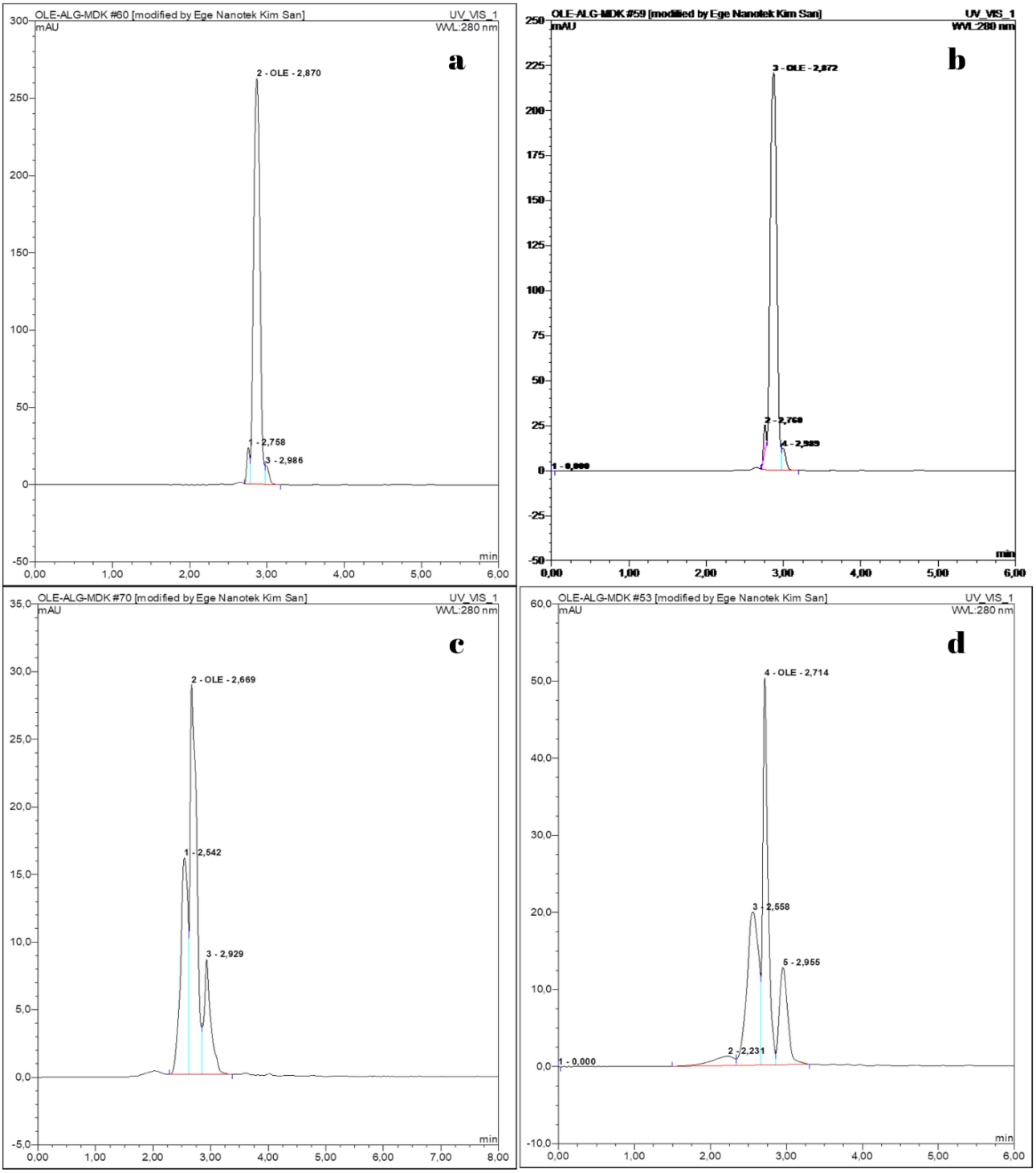

Figure 9. HPLC chromatograms of microalgae growth medium a) initially for growth under white light b) initially for growth under red light c) after growth under white light d) after growth under red light.

3.6. Determination of antioxidant capacity and total phenolic content

Antioxidant capacity and total phenolic content of the OLE used during microalgae growth are given in Table 4.

Table 4. Antioxidant capacity and total phenolic content of olive leaf extract used for microalgae growth.

	Antioxidant capacity mM Trolox/mg extract	**Total phenolic content mM GAE/mg extract**
OLE	8.984	24.074

Antioxidant capacity and total phenolic content of extracts obtained from microalgae biomass and samples taken from the growth media are given in Table 5.

Table 5. Antioxidant capacity and total phenolic content of samples taken from the growth media.

Light type, State	**Antioxidant capacity** (mM Trolox)	**Total phenolic content** (mM GAE)
White light, Initial	4.074	3.011
White light, Final	12.176	25.301
Red light, Initial	5.196	3.762
Red light, Final	10.616	10.256

As seen from Table 5, for the growth of microalgae in the presence of OLE under white light initial antioxidant capacity and total phenolic content of the growth medium were determined as 4.074 mM Trolox and 3.011 mM GAE, respectively. Although oleuropein content decreased as detected with HPLC results after completion of the growth under both white and red light, both antioxidant capacity and total phenolic content of the growth medium increased significantly. An increase in the antioxidant capacity and phenolic content during the microalgae growth in the presence of OLE can be explained by the production of bioactive compounds (De Morais et al., 2015).

This result can be interpreted as possible utilization of the phenolic compounds as a source by microalgae, in other words, biotransformation of phenolic compounds by microalgae (Papazi et al., 2017).

4. Conclusions

This study revealed that the utilization of phenolic compounds during microalgae growth is possible. The light type directly changed both the growth rate and utilization of OLE by the microalgae. With microalgae growth under both white and red light, the olive leaf extract amount in the growth medium decreased while the medium's antioxidant capacity increased. This was attributed to the production of bioactive compounds as a result of the biotransformation of polyphenols by microalgae.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by undergraduate students Ören, Yıldız, PhD student Köse and MSc student Kübra Potuk. The first draft of the manuscript was written by Köse and Dr. Bayraktar. Dr. Bayraktar and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

Abdel-Karim, O. H., Gheda, S. F., Ismail, G. A., & Abo-Shady, A. M. (2019). Phytochemical screening and antioxidant activity of Chlorella vulgaris. *Drug Invention Today*, *11*(8), 1803–1806.

Altıok, E., Bayçın, D., Bayraktar, O., & Ülkü, S. (2008). Isolation of polyphenols from the extracts of olive leaves (Olea europaea L.) by adsorption on silk fibroin. *Separation and Purification Technology*, *62*(2), 342–348.

Borowitzka, M. A. (2013). High-value products from microalgae-their development and commercialisation. In *Journal of Applied Phycology*, *25*(3), 743–756.

Castro, B. F. M., Fulgêncio, G. de O., Domingos, L. C., Cotta, O. A. L., Silva-Cunha, A., & Fialho, S. L. (2020). Positively charged polymeric nanoparticles improve ocular penetration of tacrolimus after topical administration. *Journal of Drug Delivery Science and Technology*, *60*, 101912.

De Morais, M. G., Vaz, B. D. S., De Morais, E. G., & Costa, J. A. V. (2015). Biologically Active Metabolites Synthesized by Microalgae. *BioMed Research International*, *2015*.

Della Greca, M., Pinto, G., Pistillo, P., Pollio, A., Previtera, L., & Temussi, F. (2008). Biotransformation of ethinylestradiol by microalgae. *Chemosphere*, *70*(11), 2047–2053.

Köse, M. D., Bayraktar, O., Ak B., & Atak, E. (2019). Production of Chlorella sp. in a Designed Photobioreactor. *Celal Bayar University Journal of Science*, *15*(4), 377-383.

Dilek (Yalcin), D., Udoh, U. A., Ozer (Baykal), T., Akbulut, A., Erkaya (Acikgoz), I., Yildiz, K., & Guler, D. (2012). Fourier transform infrared (FTIR) spectroscopy for identification of Chlorella vulgaris Beijerinck 1890 and Scenedesmus obliquus (Turpin) Kützing 1833. *African Journal of Biotechnology*, *11*(16), 3817–3824.

El-Sheekh, M. M., Ghareib, M. M., & Abou-El-Souod, G. W. (2012). Biodegradation of phenolic and polycyclic aromatic compounds by some algae and cyanobacteria. *Journal of Bioremediation and Biodegradation*, *3*(1).

Ferro, L., Gojkovic, Z., Gorzsás, A., & Funk, C. (2019). Statistical methods for rapid quantification of proteins, lipids, and carbohydrates in nordic microalgal species using ATR–FTIR spectroscopy. *Molecules*, *24*(18).

Gatamaneni Loganathan, B., Orsat, V., Lefsrud, M., & Wu, B. Sen. (2020). A comprehensive study on the effect of light quality imparted by light-emitting diodes (LEDs) on the physiological and biochemical properties of the microalgal consortia of Chlorella variabilis and Scenedesmus obliquus cultivated in dairy wastewater. *Bioprocess and Biosystems Engineering*, *43*(8), 1445–1455.

Góral, I., & Wojciechowski, K. (2020). Surface activity and foaming properties of saponin-rich plants extracts. In *Advances in Colloid and Interface Scienc*e, *279*, 102145.

Hernández, E., Lobato-Benítez, C., & Hernández, C. (2017). Algal enzymes, biotechnological potential uses: A review Enzimas algales, usos biotecnológicos potenciales: Una revisión. *Cymbella*, *3*, 1–15.

Jerez-Martel, I., García-Poza, S., Rodríguez-Martel, G., Rico, M., Afonso-Olivares, C., & Gómez-Pinchetti, J. L. (2017). Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. *Journal of Food Quality*, *2017*.

Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. In *Microbial Cell Factories*, *17*(1), 36.

Lee, O. H., & Lee, B. Y. (2010). Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract. *Bioresource Technology*, *101*(10), 3751–3754.

Lindner, A. V., & Pleissner, D. (2019). Utilization of phenolic compounds by microalgae. In *Algal Research*, *42*, 101602.

Matthijs, H. C. P., Balke, H., Van Hes, U. M., Kroon, B. M. A., Mur, L. R., & Binot, R. A. (1996). Application of light-emitting diodes in bioreactors: Flashing light effects and energy economy in algal culture (Chlorella pyrenoidosa). *Biotechnology and Bioengineering*, *50*(1), 98–107.

Metsoviti, M. N., Papapolymerou, G., Karapanagiotidis, I. T., & Katsoulas, N. (2019). Comparison of growth rate and nutrient content of five microalgae species cultivated in greenhouses. *Plants*, *8*(8).

Nzayisenga, J. C., Farge, X., Groll, S. L., & Sellstedt, A. (2020). Effects of light intensity on growth and lipid production in microalgae grown in wastewater. *Biotechnology for Biofuels*, *13*(1), 4.

Papazi, A., Ioannou, A., Symeonidi, M., Doulis, A. G., & Kotzabasis, K. (2017). Bioenergetic strategy of microalgae for the biodegradation of tyrosol and hydroxytyrosol. *Zeitschrift Fur Naturforschung - Section C Journal of Biosciences*, *72*(5–6), 227–236.

Pinto, G., Pollio, A., Previtera, L., Stanzione, M., & Temussi, F. (2003). Removal of low molecular weight phenols from olive oil mill wastewater using microalgae. *Biotechnology Letters*, *25*(19), 1657–1659.

Pistorius, A. M. A., DeGrip, W. J., & Egorova-Zachernyuk, T. A. (2009). Monitoring of biomass composition from microbiological sources by means of FT-IR spectroscopy. *Biotechnology and Bioengineering*, *103*(1), 123–129.

Rajurkar, N., Hande, S., & e. (2011). Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *Indian Journal of Pharmaceutical Sciences*, *73*(2), 146.

Reymann, T., Kerner, M., & Kümmerer, K. (2020). Assessment of the biotic and abiotic elimination processes of five micropollutants during cultivation of the green microalgae Acutodesmus obliquus. *Bioresource Technology Reports*, *11*, 100512.

Şahin, S., Bilgin, M., & Dramur, M. U. (2011). Investigation of Oleuropein Content in Olive Leaf Extract Obtained by Supercritical Fluid Extraction and Soxhlet Methods. *Separation Science and Technology*, *46*(11), 1829–1837.

Sarian, F. D., Rahman, D. Y., Schepers, O., & van der Maarel, M. J. E. C. (2016). Effects of Oxygen Limitation on the Biosynthesis of Photo Pigments in the Red Microalgae Galdieria sulphuraria Strain 074G. *PLOS ONE*, *11*(2), e0148358.

Somerville, V., Moore, R., & Braakhuis, A. (2019). The Effect of Olive Leaf Extract on Upper Respiratory Illness in High School Athletes: A Randomised Control Trial. *Nutrients*, *11*(2), 358.

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*, *101*(2), 87–96.

Tang, D. Y. Y., Khoo, K. S., Chew, K. W., Tao, Y., Ho, S. H., & Show, P. L. (2020). Potential utilization of bioproducts from microalgae for the quality enhancement of natural products. In *Bioresource Technology*, 304, 122997.

Yan, C., Zhao, Y., Zheng, Z., & Luo, X. (2013). Effects of various LED light wavelengths and light intensity supply strategies on synthetic high-strength wastewater purification by Chlorella vulgaris. *Biodegradation*, *24*(5), 721–732.

Yuvraj, Vidyarthi, A. S., & Singh, J. (2016). Enhancement of Chlorella vulgaris cell density: Shake flask and bench-top photobioreactor studies to identify and control limiting factors. *Korean Journal of Chemical Engineering*, *33*(8), 2396–2405.