Edaphic algae as sustainable biofactories for high-valued nutraceutical and pharmaceutical products

Hoda A. Mansour, Neamat H. El-Tablawy, Abd El-Salam M. Shaaban
Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt

Corresponding author: Neamat H. El-Tablawy, Email: neamat.hassan@sci.asu.edu.eg. Communicated by Prof. Mostafa El-Sheekh, Editor in Chief

ABSTRACT: Our present-day knowledge of the phytochemical characterization of edaphic microalgae is still largely limited due to the difficult challenges of isolation and identification. In this investigation, we highlighted the significant values of phytochemical compounds in four different edaphic algal species (*Vaucheria geminata*, *Pleurochloris pyrenoidosa*, *Botrydiopsis eriensis*, and *Scenedesmus obliquus*). Results revealed 17 different amino acids in all the investigated algae with dominance of arginine, glutamic acid and alanine as non-essential and Leucine was the dominant essential amino acids. Maximum content of amino acids was in *P. pyrenoidosa* with the highest value of essential amino acids (33.28 %). Furthermore, the ratio of essential to non-essential amino acids was nearly 1 in all algal taxa. High performance liquid chromatography (HPLC) analysis for sugars showed that mannose was the dominant sugar in both *V. geminata* and *B. eriensis* whilst arabinose and rhamnose constituted the major sugars in *P. pyrenoidosa* and *S. obliquus*, respectively. By using Gas chromatography–mass spectrometry (GC-MS), 21 different fatty acids methyl esters were identified in all algal taxa with dominance of C16/C18 types. Note of worth, polyunsaturated fatty acids were only detected in the three yellow green algae where the percentage of unsaturated fatty acids were greater than the saturated one with ratio up to 12.8 in *B. eriensis*. Dominant fatty acids identified were palmitic acid in *V. geminata*, linoleic acid in *P. pyrenoidosa*, ω−3 α-linolenic acid in *B. eriensis*, and ω−9 oleic acid in *S. obliquus*. Considering the above characteristic amino acids, fatty acids and sugars of algal taxa, we can suggest them for possible utilization in food, feed and pharmaceutical industries.

Keywords: Application, Amino acids, Edaphic algae, Fatty acids, Phytochemical components

INTRODUCTION

Algae are a diverse group of photosynthetic organisms representing the most abundant primary producers among all living organisms. They are usually inhabitants of aquatic biotopes either freshwater or marine habitats. Although they are widespread in a wide range of ecological habitats including air, soil or even extreme habitats such as hot springs, deserts or cold regions (Round, 1984). Algae that occur in terrestrial habitats including either in or on soil surfaces are called edaphic algae where they occur as free living or as resting spores (Metting, 1981). The characterization of terrestrial algal species is important for exploring their diversity and revealing their phytochemical composition. They have the ability of long survival over a long time and are adapted to adverse conditions such as xeric habitats (Gupta and Agrawal, 2006; Nayaka *et al*., 2017). To cope with different environmental conditions, algae synthesize and accumulate diverse phytochemical compounds with wide applications in all fields (Rai and Gaur, 2012; Yang *et al*., 2023). The advantages of using microalgae as a feedstock source are rapid growth with minimal nutritional requirements and high biomass content (Osorio-Reyes *et al* 2023). Besides, microalgae do not compete for cultivated land and can be grown in wastewater. Microalgae have different physiological, biochemical and molecular strategies providing them with high biomass and lipid production (Osundeko *et al*., 2019; El-Sheekh *et al*., 2023). They constitute promising organisms for investigations of secondary metabolites with medical and pharmaceutical importance (Bhattacharjee, 2016; Senousy *et al*., 2020). Microalgae are widely used in nutrition worldwide and are an important source of amino acids, carbohydrates, and fatty acids. Microalgae have great nutritional properties not only in food production for humans but also as feedstock for domestic animals (Hashemian *et al*., 2019; Williamson *et al*., 2024). Microalgae are utilized severally in poultry production and aquaculture. In which algal protein is used to improve the immune system, lipid metabolism and reproductive performance (Świątkiewicz *et al*., 2015; Ritu *et al*., 2023). Fatty acids (FAs) are a substantial part of lipids divided into several groups with respect to their structure, physiological role and biological effects (Nelson and Cox, 2008). Algal lipids represent major dietary constituents for primary consumers where they are a source of energy and essential nutrients (Rösch *et al*., 2019; Kumar *et al*., 2021).

Amino acids composition is very important in determining differences in the nutritional value of microalgal species and the quality of protein depends especially on its essential amino acid content (Haque *et al*., 2016). They are essential for the growth of all living organisms, providing the required protein and energy (Shah *et al*., 2018). On the other hand, carbohydrates can make up a large percentage of the total biomass of algae and mostly serve as storage and structure molecules. Their cell walls contain a variety of different intracellular and extracellular

polysaccharides which represent bioactive materials in pharmaceutical, nutritional and cosmetics applications (Colusse *et al*., 2022; Tyagi and Sarma, 2024).

The study of edaphic algae is important for revealing their phytochemical constituents as well as exploiting them in different applications. They are considered as promising natural sources for different valuable compounds. The objective of the present investigation was to assess the significance of some phytochemical compounds from edaphic algae for further incorporation into human nutrition, animal feeding or in pharmaceutical industries.

MATERIALS AND METHODS

Sample collection and preparation

Four edaphic algal samples were selected to perform some phytochemical analyses where obtained from the Phycology lab Botany Department Faculty of Science Ain Shams University and identified according to our previous work El-Tablawy *et al* (2020). The investigated algae include one on soil alga *Vaucheria geminata* (Vaucher) De Candolle as well, as three axenic algae isolated according to Anderson (2005) including two yellow-green algae (*Pleurochloris pyrenoidosa* Pasch and *Botrydiopsis eriensis* Snow) and one green alga (*Scenedesmus obliquus* Turpin Kütz). Each isolate was cultivated in a sterilized BG 11 media for further mass culture and incubated under continuous light intensity and 24 ±1°C temperature for two weeks. After that, algal cultures were harvested by centrifugation and preserved dry at room temperature until phytochemical analyses.

Algal growth

The growth curve for each of the three algal isolates was evaluated by measuring the optical density (OD) daily at 680 nm using Unico 1201 spectrophotometer during exponential, linear and stationary phase. In addition, biomass of each alga was estimated according to the modified method of Buono *et al* (2016) at the same previous growth stages by centrifugation of 1 liter of the algal culture and then the algal pellets were dried at 80 °C until constant weight, a linear relationship between OD and dry weight (DW) was determined for each algal strain.

Amino acids analysis

For qualitative and quantitative determinations, free amino acids were extracted according to AOAC (2012). The system used for the analysis of amino acids was high performance Amino Acid analyzer (Biochrom 30) and the software used for data collection and processing is EZ Chrom.

Fractions of carbohydrates by High Performance Liquid Chromatography (HPLC)

Chromatographic separation was accomplished using HPLC device (GBC, Australia) on a KROMASIL column (150 mm × 4.6 mm) with a UV/Vis detector and LC 1110 Pump. The HPLC analysis was performed using acetonitrile: water (75: 25 v/v) as mobile phase with a flow rate 1 ml/min and chromatograms were registered at UV 195 nm. The identification of carbohydrate compounds was accomplished by comparing their retention times with those of pure standards. Quantitative evaluation and consequent calculation were performed using Win Chrome Chromatography Ver. 1.3 software.

Analysis of fatty acids by Gas Chromatography-mass spectrophotometer (GC-MS)

A modified method of Folch *et al* (1957) was used to extract the lipids of algal samples several times with chloroform/methanol (2:1). Preparation of fatty acid methyl esters (FAMEs) from lipid extract was performed using commercial aqueous HCl as described by Ichihara and Fukubayashi (2010). GC-MS analysis was performed using Agilent Technologies 7890B GC Systems combined with 5977A Mass Selective Detector and Capillary column HP-5MS Capillary; 30.0 m×0.25 mm ID×0.25 µm film. The carrier gas was helium at a pressure of 16.9 psi with 1 µl injection. The sample was analyzed with the column held initially for 3 min at 40°C after injection, then the temperature increased to 300°C with a 20°C/min heating ramp, with a 2 min hold. Injection was carried out in splitless mode at 300 °C. MS scan range was (*m*/*z*): 40–550 atomic mass units (AMU) under electron impact (EI) ionization (70 eV). Finally, FAMEs were identified by comparing their fragmentation pattern with NIST library.

Statistical analysis

Data are reported as mean ± standard deviation from triplicate determination. The phytochemical analysis was compared using one-way ANOVA (SPSS for Windows, version 20) to identify the significant difference between samples ($p < 0.05$) (Snedecor, 2008).

RESULTS

The stationary phase of the axenic isolated edaphic algae was reached through continuous reading of the optical density of the algal culture (650 nm) to a

constant value. From Figure 1, the isolated algae shared a similar pattern of growth stages at exponential, linear and stationary phase. Although, *B. eriensis* exhibited slightly slower growth rate than the other two algae under study. By measuring the dry biomass of each alga at the same previous growth algal stages and under the same growth conditions. Maximum biomass (up to 1g/l dwt) was evaluated for *P. pyrenoidosa* followed by *B. eriensis* while only 0.5 g/l dwt was detected in *S. obliquus* (Figure 2).

Amino acids contents

From Table 1, the maximum percentage (80.26 %) of amino acids contents was detected in *P. pyrenoidosa* with the highest value of essential amino acids (33.28 %), while *V. geminata* showed the minimum value (16.19 %). Concerning amino acids composition, results revealed the presence of 17 different amino acids in all investigated algae with different quantities

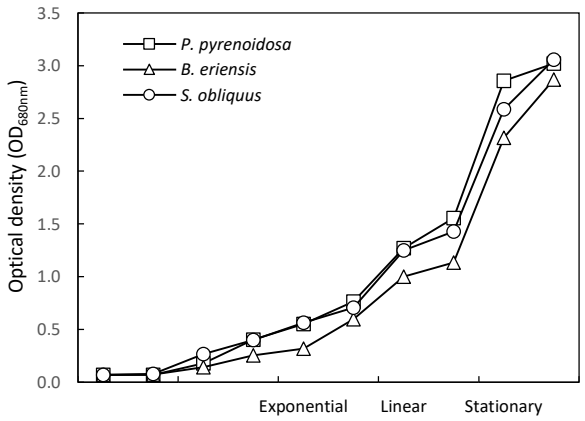

Figure 1. Growth stages of the isolated algal taxa; *P. pyrenoidosa*, *B. eriensis* and *S. obliquus*.

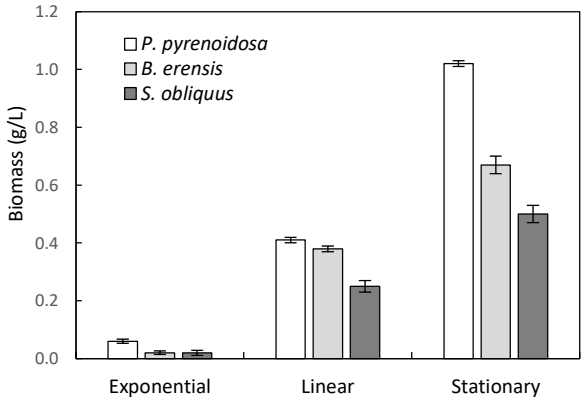

Figure 2. Biomass yield (g/l dwt) of the isolated algal taxa; *P. pyrenoidosa*, *B. eriensis* and *S. obliquus* at exponential, linear and stationary growth stage.

including 10 essential and 7 non-essential amino acids. Leucine was the dominant essential amino acids among all algal taxa under study, while cysteine and Histidine recorded the minimum values in all of them. Meanwhile, glutamic acid arginine and alanine were the most dominant non-essential amino acids. In *V. geminata* and *B. eriensis*, glutamic acid accounted for 1.88 % and 3.15 % respectively, while arginine in *P. pyrenoidosa* and alanine in *S. obliquus* accounted for 11.3 % and 3.63 % respectively. Moreover, the ratio of EAA / Non-EAA was 1 among all algal taxa under study, where the lowest value (0.71) was detected in *P. pyrenoidosa*. Also, the EAA over total amino acids was below 1 in all algae under investigation with a minimum value (0.414), especially in *P. pyrenoidosa*. The results for essential amino acids in *P. pyrenoidosa* exhibited remarkably high levels of leucine, lysine and valine (5.98, 5.05 and 4.65 % respectively) whilst the lowest value (1.45 %) was noticed for cystine. Regarding non-essential amino acids, *P. pyrenoidosa* was rich in arginine (11.3 %) as well as a high ratio of glutamic acid and alanine (8.51 and 8.38 %) whereas, the lowest content (3.4 %) was noticed for serine.

Carbohydrate composition

Results of the sugars composition of each alga analyzed with HPLC (Table 2) revealed the presence of 8 sugars in almost all algae under investigation. By comparing sugars analysis in all algae under study, *P. pyrenoidosa* showed maximum values for arabinose, glucose and galactose while mannose, fructose and sucrose recorded the highest values in *V. geminata*. On the other hand, *B. eriensis* sugars were characterized by mannose while *S. obliquus was* characterized with xylose and rhamnose.

Fatty acids composition

GC-MS analysis of methyl esters of the algal taxa revealed about 21 different fatty acids ranging from C14 to C23 (Table 3). About half of them belong to C16/C18 saturated or unsaturated fatty acids. Fatty acids are composed mainly of 6 saturated (SFAs) and 15 unsaturated fatty acids including 8 mono-unsaturated (MUFAs) and 7 polyunsaturated fatty acids (PUFAs). About 13 different fatty acids methyl esters were detected in the extract of *V. geminata*. Major fatty acids detected in the lipid extract of *V. geminata* (Figure 3) were palmitic acid (C16:0) (19.47 %) followed by the 16E-octadecenoic acid (C18:1) (7.95 %) and myristic acid (C14:0) (6.70 %).

Table 1. Essential and non-essential amino acids percentages of *V. geminata*, *P. pyrenoidosa, B. eriensis* and *S. obliquus*.

Amino acids	Side chain class	*V. geminata*	*P. pyrenoidosa*	*B. eriensis*	*S. obliquus*
Essential amino acids (EAA) %					
Cystine	Sulfur-containing	0.52^{d}±0.012	1.45^{a}±0.012	0.66^{c}±0.021	0.84^{b}±0.017
Histidine	Basic aromatic	0.35^{d}±0.015	1.61^{a}±0.018	0.73^{b}±0.018	0.41^{c}±0.006
Isoleucine	Aliphatic	0.89^{c}±0.017	2.85^{a}±0.011	1.12^{b}±0.014	1.16^{b}±0.008
Leucine	Aliphatic	1.34^{d}±0.011	5.98^{a}±0.005	2.35^{c}±0.011	2.43^{b}±0.008
Lysine	Basic	1.27^{d}±0.008	5.05^{a}±0.011	1.68^{c}±0.011	1.73^{b}±0.008
Methionine	Sulfur-containing	0.54^{d}±0.012	2.27^{a}±0.008	0.70^{c}±0.012	0.95^{b}±0.008
Phenylalanine	Aromatic	0.98^{d}±0.008	3.67^{a}±0.008	1.56^{c}±0.005	1.64^{b}±0.005
Threonine	Hydroxyl-containing	0.80^{d}±0.005	3.98^{a}±0.014	1.42^{c}±0.005	1.51^{b}±0.005
Tyrosine	Aromatic	0.72^{d}±0.005	1.77^{a}±0.011	1.22^{b}±0.008	0.90^{c}±0.011
Valine	Aliphatic	0.94^{d}±0.005	4.65^{a}±0.005	1.72^{c}±0.008	1.85^{b}±0.011
Total (EEA)		8.35	33.28	13.16	13.42
Non-essential amino acids (Non-EAA) %					
Alanine	Aliphatic	1.12^{d}±0.011	8.38^{a}±0.02	2.80^{c}±0.005	3.63^{b}±0.014
Arginine	Basic	0.86^{d}±0.008	11.30^{a}±0.008	1.52^{c}±0.014	1.75^{b}±0.014
Aspartic acid	Acid	1.76^{d}±0.014	7.10^{a}±0.008	2.48^{c}±0.008	2.71^{b}±0.02
Glutamic acid	Acid	1.88^{d}±0.012	8.51^{a}±0.005	3.15^{c}±0.017	3.50^{b}±0.014
Glycine	Aliphatic	0.87^{d}±0.014	4.67^{a}±0.012	1.59^{c}±0.017	2.28^{b}±0.014
Proline	Cyclic	0.65^{d}±0.014	3.62^{a}±0.017	1.37^{c}±0.008	1.55^{b}±0.005
Serine	Hydroxyl-containing	0.70^{d}±0.005	3.40^{a}±0.026	1.33^{c}±0.005	1.38^{b}±0.005
Total (Non-EAA)		7.84	46.98	14.24	16.8
Total AAs %		16.19	80.26	27.4	30.22
EAA/Non-EAA		1.065	0.708	0.924	0.799
EAA/Total AA		0.516	0.414	0.480	0.444

*Values are expressed as average ± standard error. Different letters indicate a significant difference at the level of p≤ 0.05.

Table 2. HPLC sugars composition in *V. geminata*, *P. pyrenoidosa*, *B. eriensis* and *S. obliquus*. Values are expressed as µg/g fwt

Retention time	Sugars name	Chemical structure	*V. geminata*	*P. pyrenoidosa*	*B. eriensis*	*S. obliquus*
3.5	Xylose	Aldose	0.72^{b}±0.008	1.32^{a}±0.012	0.44^{c}±0.003	1.33^{a}±0.011
4.7	Fructose	Ketose	1.63^{a}±0.013	0.83^{d}±0.004	1.25^{b}±0.011	0.95^{c}±0.009
5.3	Arabinose	Aldose	1.15±0.007	1.69±0.013	N.D	1.41±0.011
6.2	Mannose	Aldose	1.87^{b}±0.013	1.39^{c}±0.009	1.99^{a}±0.015	N.D
7	Galactose	Aldose	0.96^{c}±0.008	1.45^{a}±0.016	0.66^{d}±0.005	1.15^{b}±0.014
7.9	Glucose	Aldose	1.16^{c}±0.008	1.65^{a}±0.012	N.D	1.37^{b}±0.010
8.8	Sucrose	Disaccharide	1.64^{a}±0.016	1.22^{b}±0.013	1.12^{c}±0.011	0.67^{d}±0.007
10.5	Rhamnose	Deoxy sugar	1.35^{c}±0.007	1.43^{b}±0.012	N.D	1.68^{a}±0.013

*ND: not detected. Values are expressed as average ± standard error. Different letters indicate a significant difference at the level of p≤ 0.05.

Minor constituents were hypogeic acid (C16:1), 6E-hexadecenoic acid (C16:1), myristoleic acid (C14:1), 13E-octadecenoic acid (C18:1) and oleic acid (C18:1). The polyunsaturated ω-3 α-linolenic acid (C18:3), linoelaidic acid (C18:2), 13,16-octadecadienoic acid (C18:2), 10,12-tricosadiynoic acid (C23:2) and 5,8,11-eicosatriynoic acid (C20:3). Regard to *P. pyrenoidosa*, 10 fatty acids were detected. The main percentage was for the PUFA linoleic acid (C18:2) (41.72 %) followed by palmitic acid (C16:0) (29.02 %) as represented in Figure 4. While low percentage were recognized for pentadecanoic acid (C14:0), margaric acid (C17:0), behenic acid (C22:0), and lignoceric acid (C24:0) as well as minor constituents of the PUFA ω-3 α-linolenic acid (C18:3), 7,10-hexadecadienoic acid (C16:2) and the MUFA 14-octadecenoic acid (C18:1) and oleic acid (C18:1). On the other hand, only two fatty acids were detected in the chloroform: methanol extract of *B. eriensis* (Figure 5). The major portion of lipid extract (91.24 %) composed of ω-3 α-linolenic acid (C18:3) while palmitic acid (C16:0) accounted only for low percentage. Also, the extract of *S. obliquus* showed only 3 fatty acids (Figure 6) mainly composed of ω-9 oleic acid (C18:1) followed by palmitic acid (C16:0) as well as trace amount of cis-13-eicosenoic acid (C20:1). Moreover, it was noticeable from Table 3 that in all tested algae the percentage of the UFAs was greater than the SFAs with ratio of UFAs/SFAs > 1 and reach up to 12.8 in *B. eriensis*.

Table 3. Relative percentages of the fatty acids detected by GC-MS in the chloroform:methanol extracts of *V. geminata*, *P. pyrenoidosa*, *B. eriensis* and *S. obliquus*. Peak area (or percentage composition) identified are relative to other constituents within the same extract.

Fatty acids		Molecular formula	Common name	*V. geminata*	*P. pyrenoidosa*	*B. eriensis*	*S. obliquus*
Saturated FAs							
14:0	Tetradecanoic acid	$C_{14}H_{28}O_2$	Myristic acid	6.70	-	-	-
15:0	Pentadecanoic acid	$C_{15}H_{30}O_2$		-	4.12	-	-
16:0	Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid	19.47	29.02	7.12	27.37
17:0	Heptadecanoic acid	$C_{17}H_{34}O_2$	Margaric acid	-	0.55	-	-
22:0	Docosanoic acid	$C_{18}H_{34}O_2$	Behenic acid	-	0.56	-	-
24:0	Tetracosanoic acid	$C_{24}H_{48}O_2$	Lignoceric acid	-	0.56	-	-
%Sum of SFAs				26.17	34.81	7.12	27.37
Monounsaturated FAs							
14:1(n-5)	*cis*-9E-Tetradecenoic acid	$C_{14}H_{26}O_2$	Myristoleic acid	4.58	-	-	-
16:1(n-9)	*cis*-7Z-Hexadecenoic acid	$C_{16}H_{30}O_2$	Hypogeic acid	5.15	-	-	-
16:1(n-10)	6E-hexadecenoic acid	$C_{16}H_{30}O_2$		4.61	-	-	-
18:1(n-2)	16E-Octadecenoic acid	$C_{18}H_{34}O_2$		7.95	-	-	-
18:1(n-5)	13E-Octadecenoic acid	$C_{18}H_{34}O_2$		4.52	-	-	-
18:1	14-Octadecenoic acid	$C_{18}H_{34}O_2$		-	0.57	-	-
18:1(n-9)	*cis*-9Z-Octadecenoic acid	$C_{18}H_{34}O_2$	Oleic Acid	4.45	0.52	-	59.77
20:1(n-7)	*cis*-13-Eicosenoic acid	$C_{20}H_{38}O_2$	Paullinic acid	-	-	-	0.37
%Sum of MUFAs				31.26	1.09	-	60.14
Polyunsaturated FAs							
16:2(n-6)	7,10-Hexadecadienoic acid	$C_{16}H_{28}O_2$		-	2.46	-	-
18:2(n-2)	13,16-Octadecadienoic acid	$C_{18}H_{32}O_2$		4.70	-	-	-
18:2(n-6)	9E,12E-Octadecadienoic acid	$C_{18}H_{32}O_2$	Linoelaidic acid	5.52	-	-	-
18:2(n-6)	*cis,cis*-9Z,12Z-Octadecadienoic acid	$C_{18}H_{32}O_2$	Linoleic acid	-	41.72	-	-
18:3(n-3)	9Z,12Z,15Z-Octadecatrienoic acid	$C_{18}H_{30}O_2$	ω-3 α-Linolenic acid	5.52	4.45	91.24	-
20:3(n-9)	5,8,11-Eicosatriynoic acid	$C_{20}H_{28}O_2$	Mead acid	4.44	-	-	-
23:2	10,12-Tricosadiynoic acid	$C_{23}H_{38}O_2$		4.51	-	-	-
%Sum of PUFAs				24.69	48.63	91.24	-
%Sum of UFAs				56.95	49.72	91.24	60.14
%Sum of FAs				83.12	84.53	98.36	87.51
%Others				17.78	16.04	1.64	12.49
UFAs/SFAs				2.14	1.43	12.81	2.20

DISCUSSION

The nutritional quality of certain proteins is determined by the content and availability of their amino acids (Schaafsma, 2000). All essential amino acids (EAA) and non-essential amino acids (Non-EAA) were represented in all algal taxa under study. Amino acids analysis revealed that the maximum content was recorded in *P. pyrenoidosa* (about 80% dw). The most dominant amino acids were arginine, glutamic acid and alanine in comparison to other amino acids in all algae. The total EEA of *P. pyrenoidosa* was relatively high (33.28 %) in comparison to the most commonly used algal protein source *Spirulina platensis* (31.16 %) (Bashir *et al*., 2016). In addition, Arginine represented the dominant amino acid in *P. pyrenoidosa*. Previous studies of Fleurence (1999), Kakinuma *et al* (2001); Admassu, *et al* (2018) pointed that arginine, aspartic and glutamic acid were the most abundantly occurring Non-EAA in many algae while cystine typically occurs at low levels (Ursu *et al*., 2014). In addition, our results showed similar ratios of amino acids for *S. obliquus* with that of FAO/WHO (1973). Algal amino acids have many nutritional values and could be used as a supplement in a wide variety of food products with favourable effects on both energy and health (Hayes *et al.*, 2017). Microalgal peptides have also been reported by many authors to display anticancer activities (Wang and Zhang, 2013).

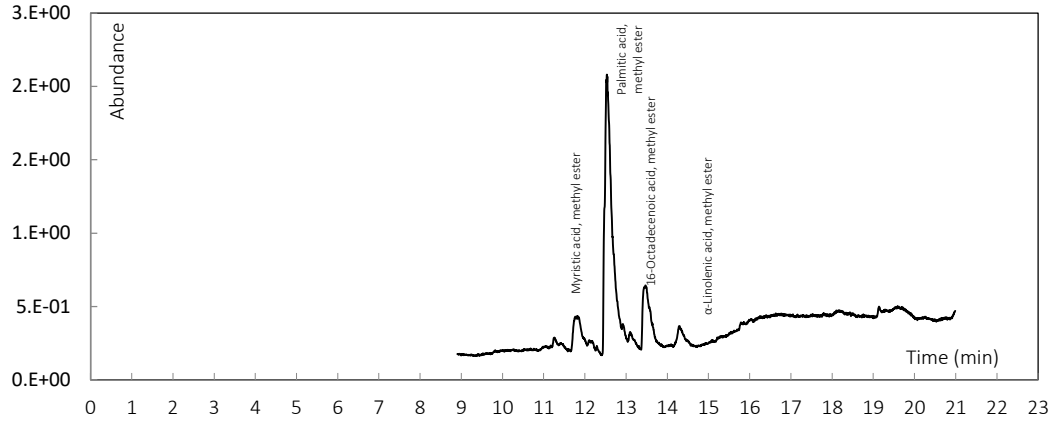

Figure 3. GC-MS chromatograph of fatty acids methyl esters in *V. geminata*.

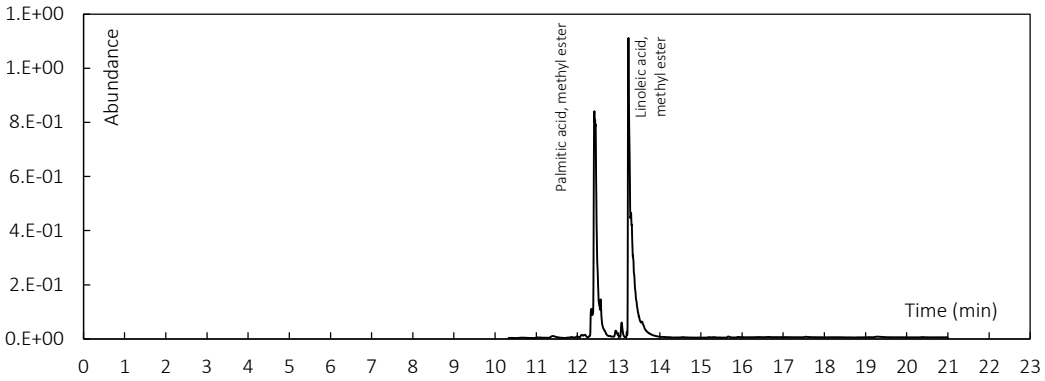

Figure 4. GC-MS chromatograph of fatty acids methyl esters in *P. pyrenoidosa*.

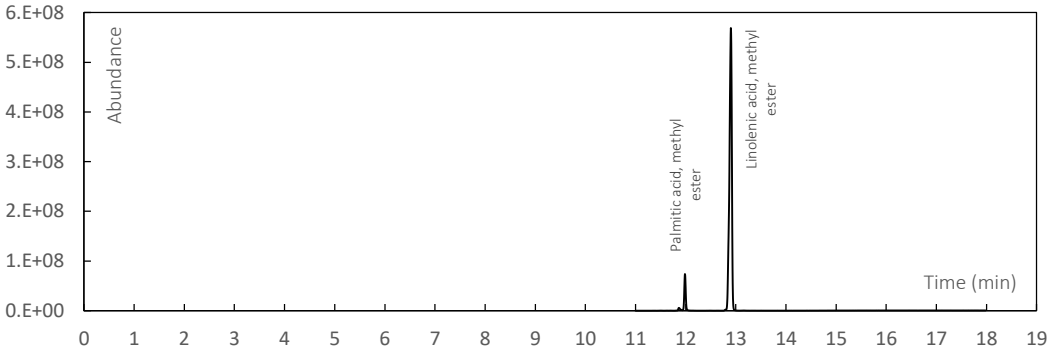

Figure 5. GC-MS chromatograph of fatty acids methyl esters in *B. eriensis*.

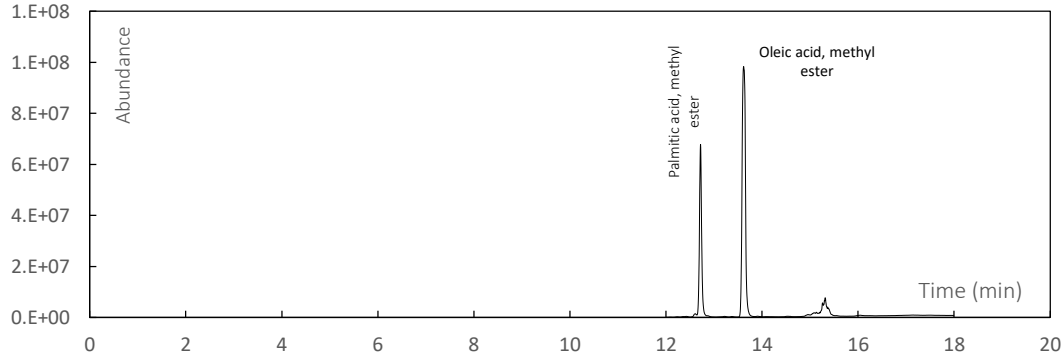

Figure 6. GC-MS chromatograph of fatty acids methyl esters in *S. obliquus*.

Arginine is considered a precursor for creatine, which plays an essential role in the energy metabolism of muscle (Khajali and Wideman, 2010) and the regulation of blood pressure (Schneider *et al*., 2015). Glutamic acid is used by almost all living organisms in the biosynthesis of their proteins, and its sodium salt is used as a food additive and flavour enhancer (Jinap and Hajeb, 2010). On the other hand, aspartic acid is a precursor of many essential amino acids (Azevedo *et al*., 2006) .

In addition, studies of Xue *et al*. (2020) exploited the adsorption characterization of aspartic acid and lysine onto nanoparticles suggesting their use in biomedical applications. Data also revealed the presence of sulphur-containing amino acids (Cystine and Methionine) in all investigated algae. Methionine is a precursor for cysteine as well as the key antioxidant glutathione (Colovic *et al*., 2018). According to Grimble (2002), methionine acts as a methyl donor in the synthesis of creatine which is essential for muscle energy generation. Moreover, the ratio of EAA to Non-EAA was nearest 1 for all studied algal taxa. That ratio is comparable to other standard reference proteins such as egg albumin, Soybean, *Chlorella* and *Spirulina* (Tibbetts *et al*., 2015) indicating that the potential use of the investigated algal taxa in animal and fish feeding. Despite the low protein content and amino acid content noticed in *V. geminata* in relative to other algal samples, a relatively higher ratio (1.07) of essential amino acids was observed indicating good quality of its protein content (Brown and Jeffrey, 1992).

To study the quality of algal carbohydrates, sugars composition of the same algal taxa was fractionated using HPLC. Glucose was not the principal sugar, instead mannose was the dominant sugar in both *V. geminata* and *B. eriensis* while arabinose and rhamnose for *P. pyrenoidosa* and *S. obliquus*. While other sugars such as xylose, fructose, sucrose and galactose were detected in varying proportions for each alga. In agreement, the study of Aspinall (2014) on the carbohydrate hydrolysate of the yellow-green alga *Tribonema equale*, yielded the same complex mixture of sugars. Works of Singab *et al* (2018) revealed the antiviral, cytotoxic, antioxidant and anti-cholinesterase properties of *S. obliquus* polysaccharides. According to Brown (1991), the nutritional quality may be related to the proportion of glucose in the hydrolysable carbohydrate of algae. Results indicated that *P. pyrenoidosa*, had the highest glucose content (1.65µg/g Fwt) of all tested algae, so it might be of high nutritional quality.

Moreover, high values of the monosaccharide mannose were detected among all sugars, reaching their maximum in *B. eriensis* and *V. geminata*. Mannose is characterised by nutritional and health-beneficial value and is used as a food supplement, cosmetics and the synthesis of Mannitol (Morita *et al*., 2013; Hu *et al*., 2016). Arabinose recorded also high values after mannose, especially in *P. pyrenoidosa*. Arabinose is commercialized as a sweetener and inhibitor of sucrase and is usually used as a food additive for diabetic patients (Inoue *et al*., 2000). Rhamnose recorded its greatest value (1.68) in the sugar constituents of the green alga *S. obliquus*. Similarly, in a previous study of Abo-Shady *et al* (1993) rhamnose was the common sugar of some green algae.

Fatty acids analysis with GC-MS of algae under investigation revealed remarkable difference in algal fatty acids profiles with a predominance of C16/C18 FAs where palmitic, oleic, linoleic, and linolenic acid were the most abundant FAs. Fatty acids vary widely among algal taxa, groups, growth stages as well as with environmental conditions. However, changing algal culture conditions and harvesting at specific growth phases may enable different FA composition of microalgal culture (Maltsev and Maltseva, 2021). Noticeably, the lipid extract of *V. geminata* recorded a wide range of fatty acids (C14- C23). The presence of long-chain FAs in the extract of *V. geminata* may be a taxonomic trait of this alga or as a precursor for long-chain hydrocarbons (Zhila *et al*., 2001). Palmitic acid was the dominant FA in addition to considerable values of 16-octadecenoic acid and myristic acid which agreed with studies of Orhan *et al* (2003) on *V. sessilis*. Palmitic acid is a saturated fatty acid with antioxidant and antimicrobial activities and strong activity against cancer cells (Ertas *et al*., 2016; Bharath *et al*., 2021).

Lipid extract of *V. geminata* revealed also minor constituents of MUFA including myristoleic acid, 7-hexadecenoic, and oleic acid as well as PUFA such as linoelaidic acid and α-linolenic acid. Myristoleic acid is an ω5 uncommon FA which conferred protection against cardiovascular diseases (Schwingshackl and Hoffmann, 2012). Also, 7-hexadecenoic acid is an uncommon ω9 FA described by Balsinde (2017) with its anti-inflammatory characteristics was detected only in the lipid extract of the studied *V. geminata*. On the other hand, linoleic acid followed by palmitic acid was the dominant FA in the isolated yellow-green alga *P. pyrenoidosa*. Linoleic acid is PUFA ω-6 fatty acid representing one of the two essential fatty acids for

humans which must be obtained through their diet and has great potential in human nutrition (Jandacek, 2017). Besides, the rare ω-6 fatty acid 7,10-hexadecadienoic acid was observed as a minor constituent in the lipid profile of *P. pyrenoidosa*. Which was evaluated for its antimicrobial, antioxidant, and anti-inflammatory activities (Buszewski *et al*., 2019). In *B. eriensis*, only two fatty acids were detected; the major was α-linolenic acid, and the minor constituent is palmitic acid. Alpha-linolenic acid is ω-3 PUFA, which the human body must obtain from food components for good health, as well as reduces the risk of cardiovascular diseases (Yin *et al.,* 2023) . In aquaculture, better growth is obtained when marine animals fed on PUFA-rich microalgae (Vijayaram *et al*., 2024).

Yellow-green algae become the focus of intensive research due to their high lipid productivity, especially PUFAs as well as other interesting lipids (Krzemińska *et al*., 2020). In relevant to the green alga *S. obliquus*, oleic acid was the major fatty acid followed by palmitic acid. This percentage (60 %) was similar to previous studies of Abomohra *et al*., (2013) and Salama *et al* (2013). The microalgal extract containing oleic acid exhibited antimicrobial and antioxidant capabilities (Conde *et al*., 2021). Oleic acid is an ω-9 essential fatty acid which decreases the susceptibility to heart attack risk and arthrosclerosis (Macri *et al*., 2015). Results revealed also, a minor constituent of *cis*-13-eicosenoic acid in *S. obliquus* which has been found to have antimicrobial activity. The most favorable lipid profile is obtained when UFAs replace or are greater than SFAs which is associated with reduced cardiovascular disease (Djuricic and Calder, 2021). This was noticed in all algal taxa under investigation, particularly *S. obliquus*.

CONCLUSION

In conclusion, the studied edaphic microalgae have all the needed components for human nutrition and animal feeding. The ratio of essential and non-essential amino acids was ~1 among all algal taxa, where the highest value (1.07) was detected in *Vaucheria geminata*. In addition, various fractions of sugars were evaluated such as mannose, rhamnose, glucose, arabinose, galactose and fructose. Also, remarkable differences in algal fatty acids profiles were observed throughout this study. In *Vaucheria geminata* 13 fatty acids representing about 82.12 % of the total constituents were identified, only 2 of them belonging to SFAs, while 11 were from USFAs. Similarly, *Pleurochloris pyrenoidosa* contained 10 FA about 84.53 % of the total constituents, of them 5 SFAs and 5 UFAs were characterized. It was noted that only two fatty acids were detected in *Botrydiopsis eriensis* representing a large proportion (98.36 %) of the total constituents composed mainly of PUFA (ω-3 α-Linolenic acid) and low portion of palmitic acid. Also, *Scenedesmus obliquus* showed only 3 fatty acids (87.51 %) with a high ratio of MUFAs to SFAs composed mainly of ω-9 oleic acid followed by palmitic acid.

This investigation indicated that not only green algae have different and important applications in human life but also members of Xanthophycophyta will play an important applied role in the future of animal feeds, human foods, the drugs industry, as well as biodiesel production.

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Authors' contributions: AMS designed the experimental approach, analyzed the data, and helped in writing the manuscript. NHE carried out isolation of algae and all the phytochemical studies. NHE generated all the tables and figures in the manuscript. HAM helped to draft the manuscript. All authors read and approved of the final manuscript.

REFERENCES

Abo-Shady, A. M., Mohamed, Y. A., Lasheen, T. (1993). Chemical composition of the cell wall in some green algae species. *Biol. Plant.* 35(4):629–632. https://doi.org/10.1007/BF02928041

Abomohra, A. E., Wagner, M., El-Sheekh, M., Hanlet, D. (2013). Lipid and total fatty acid productivity in photoautotrophic fresh water microalgae : screening studies towards biodiesel production. *J. Appl. Phycol.* 25:931–936. https://doi.org/10.1007/s10811-012-9917-y

Admassu, H., Abera, T., Abraha, B., Yang, R., Zhao, W. (2018). Proximate, mineral and amino acid composition of dried laver (*Porphyra* spp.) Seaweed. *JAIR.* 6(9):149 – 154.

Andersen, R. A. (2005). Algal culturing techniques. Elsevier.

AOAC. (2012). AOAC official methods of analysis (18th ed.).

Gaithersburg, USA: AOAC international.

Aspinall, G. O. (2014). The polysaccharides. *Academic Press.*

Azevedo, R. A. de, Lancien, M., Lea, P. J. (2006). The aspartic acid metabolic pathway, an exciting and essential pathway in plants. *Amino Acids,* 30(2):143–162.

Balsinde, J. (2017, May). Anti-inflammatory Mechanisms of 7-cis-Hexadecenoic acid. In *Proceedings of the CIBERDEM Annual Meeting, Cerdanyola del Valles, Spain* (pp. 17-19).

Bashir, S., Sharif, M. K., Butt, M. S., Shahid, M. (2016). Functional properties and amino acid profile of *Spirulina platensis* protein isolates. *Biological Sciences-PJSIR*, 59(1):12–19.

Bharath, B., Perinbam, K., Devanesan, S., AlSalhi, M. S., Saravanan, M. (2021). Evaluation of the anticancer potential of Hexadecanoic acid from brown algae *Turbinaria ornata* on HT–29 colon cancer cells. *Journal of Molecular Structure*, *1235*, 130229.

Bhattacharjee, M. (2016). Pharmaceutically valuable bioactive compounds of algae. *Asian J Pharm Clin Res.* 7:43–47.

Brown, M. R. (1991). The amino-acid and sugar composition of 16 species of microalgae used in mariculture. *J. Exp. Mar. Biol. Ecol.* 145(1):79–99.

Brown, M. R., Jeffrey, S. W. (1992). Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 1. Amino acids, sugars and pigments. *J. Exp. Mar. Biol. Ecol.* 161(1):91–113. https://doi.org/https://doi.org/10.1016/0022-0981(92)90192-D

Buono, S., Colucci, A., Angelini, A., Langellotti, A. L., Massa, M., Martello, A, … Dibenedetto, A. (2016). Productivity and biochemical composition of *Tetradesmus obliquus* and *Phaeodactylum tricornutum*: effects of different cultivation approaches. *J. Appl. Phycol.* 28:3179–3192. https://doi.org/10.1007/s10811-0876-6

Buszewski, B., Rafińska, K., Cvetanovic, A., Walczak, J., Krakowska, A., Rudnicka, J., Zekovic, Z. (2019). Phytochemical analysis and biological activity of *Lupinus luteus* seeds extracts obtained by supercritical fluid extraction. *Phytochem. Lett.* https://doi.org/10.1016/j.phytol.2019.02.014

Colovic, M. B., Vasic, V. M., Djuric, D. M., Krstic, D. Z. (2018). Sulphur-containing amino acids: protective role against free radicals and heavy metals. *Curr. Med. Chem*. 25(3):324–335.

Colusse, G. A., Carneiro, J., Duarte, M. E. R., Carvalho, J. C. D., Noseda, M. D. (2022). Advances in microalgal cell wall polysaccharides: a review focused on structure, production, and biological application. *Critical Reviews in Biotechnology*, *42*(4), 562-577.

Conde, T. A., Neves, B. F., Couto, D., Melo, T., Neves, B., Costa, M., Domingues, M. R. (2021). Microalgae as sustainable bio-factories of healthy lipids: Evaluating fatty acid content and antioxidant activity. *Marine drugs*, *19*(7), 357.

Djuricic, I., and Calder, P. C. (2021). Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: An update for 2021. *Nutrients*, *13*(7), 2421.

El-Sheekh, M. M., Galal, H. R., Mousa, A. S. H., Farghl, A. A. (2023). Coupling wastewater treatment, biomass, lipids, and biodiesel production of some green microalgae. *Environmental Science and Pollution Research*, *30*(12), 35492-35504.

El-Tablawy, N. H., Mansour, H. A., Shaaban, A. M. (2020). Antioxidant activities of some edaphic algae in Egypt. *BJBAS.* 9(1):1–11.

Ertas, A., Boga, M., Gazioglu, I., Yesil, Y., Hasimi, N., Ozaslan, C., Kaplan, M. (2016). Fatty acid, essential oil and phenolic compositions of *Alcea pallida* and *Alcea apterocarpa* with antioxidant, anticholinesterase and antimicrobial activities. *Chiang Mai J. Sci.* 43(1):89–99.

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends Food Sci Technol.* 10(1):25–28.

Folch, J., Lees, M., Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry,* 226(1), 497–509.

Grimble, R. F. (2002). Sulphur amino acids, glutathione and immune function. In *Nutrition and immune function* (pp. 133-150).

Gupta, D., Kumar, M. (2017). Evaluation of in vitro antimicrobial potential and GC–MS analysis of *Camellia sinensis* and *Terminalia arjuna*. *Biotechnol. Rep*. 13:19–25. https://doi.org/https://doi.org/10.1016/j.btre.2016.11.002

Gupta, S., Agrawal, S. C. (2006). Survival of blue-green and green algae under stress conditions. *Folia Microbiologica* 51(2):121–128.

Haque, M. A., Timilsena, B. Y. P., Adhikari, B. (2016). Food proteins, structure, and function. Reference Module in Food Science. Http://Dx. Doi. Org/10.1016/B978-0-08-100596-5.03057-2.

Hashemian, M., Ahmadzadeh, H., Hosseini, M., Lyon, S., Pourianfar, H. R. (2019). Production of microalgae-derived high-protein biomass to enhance food for animal feedstock and human consumption. In Advanced Bioprocessing for Alternative Fuels, Biobased Chemicals, and Bioproducts (pp 393–405).

Hayes, M., Skomedal, H., Skjånes, K., Mazur-Marzec, H., Toruńska-Sitarz, A., Catala, M., García-Vaquero, M. (2017). Microalgal proteins for feed, food and health. In Microalgae-based biofuels and bioproducts (pp. 347–368). http://dx.doi.org/10.1016/B978-0-08-101023-5.00015-7

Hu, X., Shi, Y., Zhang, P., Miao, M., Zhang, T., Jiang, B. (2016). D-Mannose: Properties, production, and applications: An overview. *Compr. Rev. Food Sci. Food Saf.* 15(4):773–785.

Ichihara, K., Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography. *J. Lipid Res.* 51(3): 635–640.

Inoue, S., Sanai, K., Seri, K. (2000). Effect of L-arabinose on

blood glucose level after ingestion of sucrose-containing food in human. *J JPN SOC FOOD SCI.* 53(6):243–247.

Jandacek, R. J. (2017). Linoleic acid: a nutritional quandary. *Healthcare 2017,* 5, 25; Multidisciplinary Digital Publishing Institute. doi:10.3390/healthcare5020025

Jinap, S., Hajeb, P. (2010). Glutamate. Its applications in food and contribution to health. *Appetite,* 55(1):1–10.

Joint FAO, Organization WH (1973) Energy and protein requirements: report of a Joint FAO/WHO ad hoc expert committee [meeting held in Rome from 22 March to 2 April 1971]. Energy and Protein Requirements: Report of a Joint FAO/WHO Ad Hoc Expert Committee [Meeting Held in Rome from 22 March to 2 April 1971].

Kakinuma, M., Park, C. S., Amano, H. (2001). Distribution of free L-cysteine and glutathione in seaweeds. *Fish Sci* . 67(1):194–196.

Khajali, F., Wideman, R. F. (2010) Dietary arginine: metabolic, environmental, immunological and physiological interrelationships. *Poult. Sci. J.* 66(4):751–766.

Krzemińska, I., Nosalewicz, A., Reszczyńska, E., Pawlik-Skowrońska, B. (2020). Enhanced light-induced biosynthesis of fatty acids suitable for biodiesel production by the yellow-green alga Eustigmatos magnus. *Energies*, *13*(22), 6098.

Kumar, B. R., Mathimani, T., Sudhakar, M. P., Rajendran, K., Nizami, A. S., Brindhadevi, K., Pugazhendhi, A. (2021). A state of the art review on the cultivation of algae for energy and other valuable products: application, challenges, and opportunities. *Renewable and Sustainable Energy Reviews*, *138*, 110649.

Macri, E. V., Lifshitz, F., Alsina, E., Juiz, N., Zago, V., Lezón, C., Friedman, S. M. (2015). Monounsaturated fatty acids-rich diets in hypercholesterolemic-growing rats. *Int J Food Sci Nutr* . 66(4):400–408.

Maltsev, Y., & Maltseva, K. (2021). Fatty acids of microalgae: Diversity and applications. *Reviews in Environmental Science and Bio/Technology*, *20*, 515-547.

Metting, B. (1981) The systematics and ecology of soil algae. *The Botanical Review.* 47(2):195-312.

Morita, T., Fukuoka, T., Imura, T., Kitamoto, D. (2013). Production of mannosylerythritol lipids and their application in cosmetics. *Appl. Microbiol. Biotechnol.* 97(11):4691–4700.

Nayaka, S., Toppo, K., Verma, S. (2017). Adaptation in Algae to Environmental Stress and Ecological Conditions. In Plant Adaptation Strategies in Changing Environment (pp 103–115). Springer.

Nelson, D., Cox, M. (2008). Chapter 21: Lipid Biosynthesis. Principles of Biochemistry, 787–815.

Orhan, I., Sener, B., Atici, T. (2003). Fatty acid distribution in the lipoid extracts of various algae. *Chem. Nat. Compd.* 39(2): 167–170.

Osorio-Reyes, J. G., Valenzuela-Amaro, H. M., Pizaña-Aranda, J. J. P., Ramírez-Gamboa, D., Meléndez-Sánchez, E. R., López-Arellanes, M. E., Martínez-Ruiz, M. (2023). Microalgae-based biotechnology as alternative biofertilizers for soil enhancement and carbon footprint reduction: Advantages and implications. *Marine Drugs*, *21*(2), 93.

Osundeko, O., Ansolia, P., Gupta, S. K., Bag, P., Bajhaiya, A. K. (2019). Promises and challenges of growing microalgae in wastewater. In Water Conservation, Recycling and Reuse: Issues and Challenges (pp 29–53).

Rai, L. C., Gaur, J. P. (2012). Algal adaptation to environmental stresses: physiological, biochemical and molecular mechanisms. P 421

Rippka, R. (1992). Pasteur culture collection of cyanobacterial strains in axenic culture. Catalogue and Taxonomic Handbook, Catalogue of Strains 1992/1993, 1:1–103.

Ritu, J. R., Ambati, R. R., Ravishankar, G. A., Shahjahan, M., Khan, S. (2023). Utilization of astaxanthin from microalgae and carotenoid rich algal biomass as a feed supplement in aquaculture and poultry industry: An overview. *Journal of Applied Phycology*, *35*(1), 145-171.

Rösch, C., Roßmann, M., Weickert, S. (2019). Microalgae for integrated food and fuel production. *Gcb Bioenergy*, 11(1):326–334.

Round, F. E. (1984). The ecology of algae. CUP Archive.

Salama, E-S., Kim, H-C., Abou-Shanab, R. A. I., Ji, M-., Oh, Y-K., Kim, S-H., Jeon, B-H. (2013). Biomass, lipid content, and fatty acid composition of freshwater *Chlamydomonas mexicana* and *Scenedesmus obliquus* grown under salt stress. *Bioprocess Biosyst Eng*. 36(6):827–833.

Schaafsma G (2000) The protein digestibility–corrected amino acid score. J. Nutr. 130(7):1865S-1867S.

Schneider, J. Y., Rothmann, S., Schröder, F., Langen, J., Lücke, T., Mariotti, F., Tsikas, D. (2015). Effects of chronic oral L-arginine administration on the L-arginine/NO pathway in patients with peripheral arterial occlusive disease or coronary artery disease: L-Arginine prevents renal loss of nitrite, the major NO reservoir. *Amino Acids,* 47(9): 1961–1974.

Schwingshackl, L., Hoffmann, G. (2012). Monounsaturated fatty acids and risk of cardiovascular disease: synopsis of the evidence available from systematic reviews and meta-analyses. *Nutrients*, 4(12):1989–2007.

Senousy, H. H., Abd Ellatif, S., & Ali, S. (2020). Assessment of the antioxidant and anticancer potential of different isolated strains of cyanobacteria and microalgae from soil and agriculture drain water. *Environmental Science and Pollution Research*, *27*, 18463-18474.

Shah, M. R., Lutzu, G. A., Alam, A., Sarker, P., Chowdhury, M. A. K., Parsaeimehr, A., Daroch, M. (2018). Microalgae in aquafeeds for a sustainable aquaculture industry. *J. Appl. Phycol.* 30(1):197–213.

Singab, A. N., Ibrahim, N., Elsayed, A. E., El-Senousy, W., Aly, H., Abd Elsamiae, A., Matloub, A. A. (2018). Antiviral, cytotoxic, antioxidant and anti-cholinesterase activities of polysaccharides isolated from microalgae *Spirulina platensis*, *Scenedesmus obliquus* and *Dunaliella salina*.

Arch. Pharm. Sci. 2(2):121–137.

Snedecor, G. W. (2008). In: The Concise Encyclopedia of Statistics.

Świątkiewicz, S., Arczewska-Włosek, A., Józefiak, D. (2015). Application of microalgae biomass in poultry nutrition. *Poult. Sci. J.* 71(4):663–672.

Tibbetts, S. M., Bjornsson, W. J., McGinn, P. J. (2015). Biochemical composition and amino acid profiles of *Nannochloropsis granulata* algal biomass before and after supercritical fluid CO_2 extraction at two processing temperatures. *Anim. Feed Sci. Technol.* 204:62–71.

Tyagi, U., Sarma, A. K. (2024). Bioactive Compounds from Algae: Extraction, Purification, and Applications. In *Value Added Products From Bioalgae Based Biorefineries: Opportunities and Challenges* (pp. 259-288). Singapore: Springer Nature Singapore.

Ursu, A-V., Marcati, A., Sayd, T., Sante-Lhoutellier, V., Djelveh, G., Michaud, P. (2014). Extraction, fractionation and functional properties of proteins from the microalgae *Chlorella vulgaris*. *Bioresour. Technol.* 157:134–139.

Vijayaram, S., Ringø, E., Ghafarifarsani, H., Hoseinifar, S. H., Ahani, S., & Chou, C. C. (2024). Use of Algae in Aquaculture: A Review. *Fishes*, *9*(2), 63.

Wang, X., Zhang, X. (2013). Separation, antitumor activities, and encapsulation of polypeptide from *Chlorella pyrenoidosa*. *Biotechnol. Prog.* 29(3):681–687.

Williamson, E., Ross, I. L., Wall, B. T., Hankamer, B. (2024). Microalgae: Potential novel protein for sustainable human nutrition. *Trends in Plant Science*, *29*(3), 370-382.

Xue, M., Sampath, J., Gebhart, R., Haugen, H. J., Lyngstadaas, S. P., Pfaendtner, J., Drobny, G. P. (2020). Studies of dynamic binding of amino acids to TiO_2 nanoparticle surfaces by Solution NMR and Molecular Dynamics Simulations. Langmuir.

Yang, Y., Hassan, S. H., Awasthi, M. K., Gajendran, B., Sharma, M., Ji, M. K., & Salama, E. S. (2023). The recent progress on the bioactive compounds from algal biomass for human health applications. *Food Bioscience*, *51*, 102267.

Yin, S., Xu, H., Xia, J., Lu, Y., Xu, D., Sun, J., Sun, G. (2023). Effect of alpha-linolenic acid supplementation on cardiovascular disease risk profile in individuals with obesity or overweight: A systematic review and meta-analysis of randomized controlled trials. *Advances in Nutrition*, *14*(6), 1644-1655.

Zeh, K., Chang, J. (2011). Nitrogen starvation strategies and photobioreactor design for enhancing lipid content and lipid production of a newly isolated microalga *Chlorella vulgaris* ESP-31: Implications for biofuels. *Biotechnol. J.* 6(11):1358–1366.

Zhila, N. O., Kalacheva, G. S., Volova, T. G., Degermendzhi, A. G. (2001). Structure of hydrocarbons synthesized by the alga *Botryococcus* Isolated from Lake Shira. Dokl. *Biol. Sci.* 378(1):265–269.