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Research Article

Fatty Acid Profiles, Pigments and Biochemical Contents of Cyanobacterial Mat in Some Lakes of North Western Desert, Egypt

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Abstract

Objective: This is the first attempt to investigate the natural dens grow of cyanobacterial mats as a source of some bioactive materials and biodiesel in some northern lakes of western desert, Egypt. **Materials and Methods:** The investigation included fatty acids, pigments (chlorophyll a, -carotein and phycocyanin) and biochemical contents (glycerol, total protein, carbohydrates and lipids), in addition to the % of water and ash contents from autumn- 2013 to summer-2014. Cyanobacterial mat was important source of protein, phycocyanin, -carotene and a wide range of fatty acids. **Results:** Protein content was 284.09 wwt% in Aghormy whereas -carotene reached to 41 times more than in carrot. The detected levels of phycocyanin (PC) in wet natural growing mats of Aghormy and Maraqi were similar to that reported in optimized dry mass production. Fatty acids spectrum found in the present study are very suitable for use as biodiesel especially in Aghormy where fatty acids characterized by high unsaturation and significant amount of palmitic and oleic fatty acids. Based on the fatty acids profile, seven biodiesel quality criteria were calculated; Cetane Number (CN), the Mass of Iodine (IV), Saponification Value (SV), the Cold Filter Plugging Point (CFPP), Long-Chain Saturated Factor (LCSF), the temperature at which the solid phase begins to form (CP) and Degree of Unsaturation (DU). Based on the biodiesel quality criteria, the best biodiesel was detected in Aghormy Lake. **Conclusion:** The cyanobacterial mats in Siwa oasis is considered as a natural resource of a wide range of commercially important natural products.

Key words: Cyanobacterial mats, fatty acids, pigments, biochemical contents, biodiesel, siwa oasis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Microbial mats are accretionary, cohesive, macroscopic accumulations of microbial communities, which are often laminated and grow mostly on submerged or moist surfaces¹. In mat, cyanobacteria are usually the most conspicuous microorganisms that have the capacity for oxygenic photosynthesis and fix atmospheric nitrogen² and they are a rich source of potentially useful natural products³.

Cyanobacteria contain a diverse array of pigments, which have tremendous potential as natural dyes, antioxidants, nutritional and pharmaceutical supplements in bio-industry^{4,5}. The extensively pigments in bio-industry, are the phycobili proteins, which account for about 20% of total dry weight of many cyanobacteria^{6,7}. Phycocyanin is an accessory photosynthetic pigment of the phycobiliprotein⁸, which has been used in pharmaceutical industries⁹. Cyanobacterial lipids are rich in essential fatty acids such as the C₁₈ linoleic and γ -linolenic acids and their C₂₀ derivatives¹⁰. These fatty acids are essential components of the diet of humans and animals and are important feed additives in aquaculture¹¹. Cyanobacteria such as *Spirulina* and *Nostoc* have been used as a source of protein and vitamin for humans and animals^{12,13}.

There is a significant interest in using microalgae to produce oil for biodiesel because of their potential for high productivity and because they can be cultivated in areas unsuitable for conventional crop production¹⁴. Microalgae already reach up to 300 times more oil productivity for biodiesel production than traditional crops on area basis^{15,16}. The variation in microalgal fatty acids composition as a response to changes in certain environmental variables forms important facet to feedstock selection¹⁷. The most promising renewable feedstock for producing third-generation biofuel next to eukaryotic microalgae is prokaryotic blue-green algae (cyanobacteria). The composition of cyanobacterial fatty acids is highly variable, like the algae themselves¹⁸. Microalgae and cyanobacteria are considered as a suitable sources of renewable liquid biofuels consisting of hydrocarbon chains that can replace petroleum hydro-carbons as fuels, lubricants, plastics, etc.¹⁹. Cyanobacteria are a promising source of biomass for production of biofuel (cyanofuel) because of their fast growth, high productivity and tolerance to genetic manipulations²⁰. Cyanobacteria microorganism considered as a source of renewable energy because they able to cope with some of the major difficulties encountered with preceding biofuel generations, furthermore, cyanobacteria offer a promising biomass feedstock for various biofuels²¹. The present study is a first-step to investigate how the dense grow of cyanobacterial mats in north-western desert can be

economically utilized. To achieve this goal, the mat constituents of total lipid, protein, carbohydrates, glycerol, chlorophyll a, phycocyanin, β -carotene and fatty acids profile were investigated in three lakes of Siwa oasis. Thereafter, the fatty acid profiles were used to attest the suitability of mats lipid as a source of biofuel.

MATERIALS AND METHODS

Sites of work: Three lakes, Aghormy, Zieton and Maraqui, in Siwa oasis were studied (Fig. 1). The lakes main features and characters were presented in details in Abd El-Karim and Goher²². Cyanobacterial mats were collected from autumn, 2013 to summer, 2014. From each lake, approximately 1 kg of the cyanobacterial mats (1-3 mm top layer) were collected from the margins of each lake, transferred on ice to the laboratory and stored at -30°C. Few days later, fresh mats were used for different analyses.

Biochemical analysis: Total protein contents were determined by Biuret method²³ using bovine albumin as stander. The hydrolysis of carbohydrate was carried out by Myklestad and Haug²⁴ and determined by the phenol sulphuric acid method as described by DuBois *et al.*²⁵ using glucose as stander. Total lipid contents were determined by the sulfophosphovanillin procedure (SPV), using cholesterol as stander²⁶. Glycerol was determined Spectrophotometrically at 410 nm and compared to calibration standards²⁷. Different biochemical contents were reported as percentage of wet weight (wwt%).

Pigments analyses: Phycocyanin was determined spectrophotometric as described by Patel *et al.*²⁸ and reported as milligram per gram wet weight. The β -carotene pigment was measured according to Davies²⁹ and compared to standard curve. The readings were reported as percentage of wet weight (wwt%). Chlorophyll a was extracted and measured according to APHA³⁰ using Perkin Elmer (LS45) fluorescence spectrometer at an excitation wavelength of 430 nm and an emission wavelength of 663 nm and compared to standard curve and reported as microgram per gram wet weight.

Water and ash contents: Known weight of sample was dried to constant weight in an oven maintained at 110°C³¹. For ash contents, a known weight of well-mixed dried sample was ignited in furnace at 600°C for 1 h. Cool in the desiccators and weight soon after it reached room temperature.

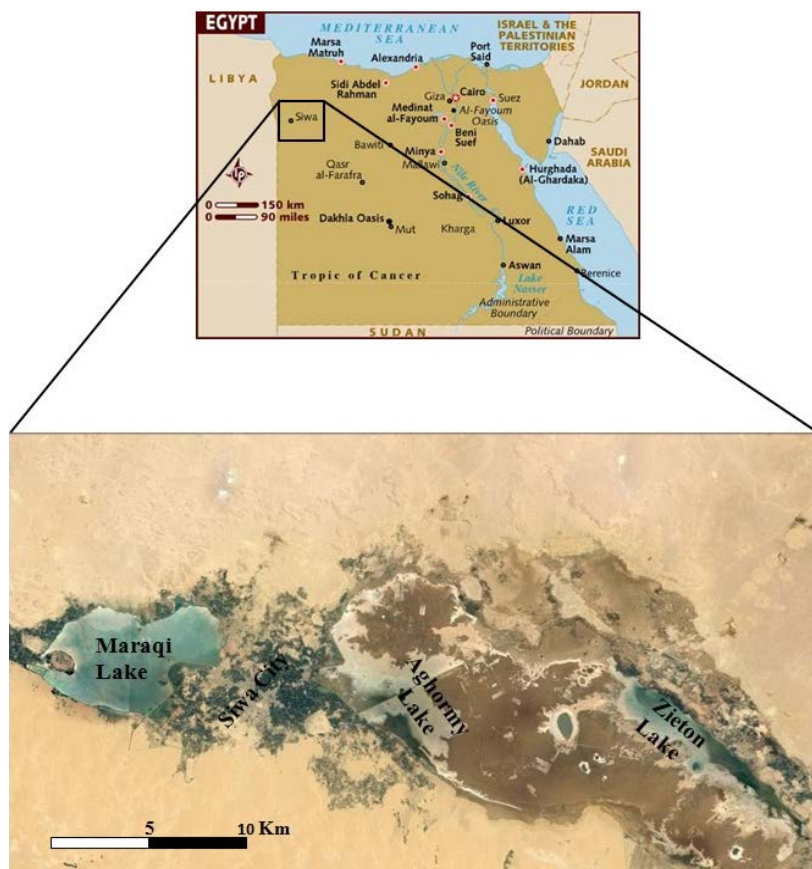


Fig. 1: Satellite image of Siwa oasis showing the three lakes of work

Fatty acids: Lyophilized mats collected in winter were used to fatty acids analysis. Extraction, methylation and quantification of freeze-dried samples were occurred using modified Folch method^{32,33}. Fatty acids were measured by Gas Chromatography Mass Spectrometry (GC-MS) using Agilent 7890 Series GC system interfaced to 5975 inert MSD with triple-axis detector MS. Extraction, methylation, quantification and measurements are presented in details in Abd El-Karim *et al.*³⁴. For fatty acids normalization, lipid content in the lyophilized mat samples was measured as presented above.

Estimation of biodiesel fuel quality criteria based on fatty acids profile:

The parameters attesting for the quality of the bio-diesel were estimated in relation to the molecular structures of fatty acids, which may vary according to carbon chain sizes and the amount and/or position of double bonds³⁵. These molecular characteristics greatly influence the main parameters of biodiesel quality such as cetane number, Iodine Value (IV), the Cold Filter Plugging Point (CFPP) and the oxidation stability³⁶.

Cetane Number (CN) is indicative of the time delay in the ignition of fuel for diesel cycle engines. The higher the CN, the shorter is the ignition time. The CN increases with the length of the unbranched carbon chain of the FAME components³⁷. The higher the carbon chain length of the methyl esters, the higher is the density and viscosity of the biodiesel, characteristics that will decrease with the increasing number of double bonds³⁶. Range of required CN for a quality biodiesel is usually 40-50³⁸; American Standards for Testing and Materials (ASTM) D675 (minimum CN of 47) and European standards EN 14214 (minimum CN of 51)³⁶.

$$CN = \frac{46.3 + 5.458}{SV} - 0.225 \times IV \quad (1)$$

The IV (the mass of iodine, in grams, that is consumed by 100 g of a chemical substance) refers to the tendency of biodiesel to react with oxygen at near ambient temperature³⁸. This characteristic depends on the number and the position of the double bonds in the carbon chains of the alkyl esters. The higher the IV, the higher the possibility of oxidation, deposits formation and deterioration of the biodiesel lubricity. The

maximum IV accepted in Europe is 120 g I2/100 g. The IV for soybean oil, in the range of 120-141 is indicative of a higher susceptibility to oxidative attack³⁹. Saponification Value (SV) refers to milligrams of potassium hydroxide required to saponify 1 g of oil, which is inversely related to the ester's molecular weight. The IV and SV are calculated using Eq. 2 and 3, respectively, according to Francisco *et al.*⁴⁰:

$$IV = \frac{\sum (254 \times DN)}{M} \quad (2)$$

$$SV = \frac{\sum (560 \times N)}{M} \quad (3)$$

where, D is the number of double bonds, M is the FA molecular mass and N is the percentage of each FA component of the microalgae oil.

The CFPP is usually used for the prediction of the flow performance of biodiesel at low temperatures^{37,41}. The standardized maximum of CFPP is 19°C⁴². The larger the size of the carbon chains or the higher degree of saturation of FAME molecules composing biodiesel, the higher will be the value of CFPP and the worse their low temperature properties⁴¹. The standards do not mention a low-temperature parameter in their lists of specifications. Equation 5 has been generated by correlating the value of the CFPP with a factor related to chains saturation and length (Long Chain Saturated Factor (LCSF)). The LCSF value was estimated through Eq. 4, applied to several oils sources, by weighing up values of the longer chains (C16, C18, C20, C22 and C24 are the weight percentages of each of the fatty acids) to reproduce their impact on the fuel cold flow properties^{40,41}. A regression, based on data of these two properties (LCSF and CFPP), defined the cetane response for different levels of saturation, with a good correlation ($R^2 = 0.97$)^{40,41}.

$$LCSF = (0.1 \times C16) + (0.5 \times C18) + (1 \times C20) + (1.5 \times C22) + (2.0 \times C24) \quad (4)$$

$$CFPP = (3.1417 \times LCSF) - 16.477 \quad (5)$$

The DU represented the chain length of each component ester and was calculated based on Eq. 6, as the amount of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA; in weight percent) present in the microalgae oil⁴³.

$$DU = MUFA + (2 \times PUFA) \quad (6)$$

The CP is defined as the temperature at which the solid phase begins to form, is another feature related to biodiesel cold flow properties and is more favorable as an industry standard than CFPP as it is more indicative of biodiesel performance in the field⁴⁴. A strong correlation ($R^2 = 0.96$) was found between CP and palmitic acid methyl ester (C16:0) content⁴³ and was reported through the following Eq. 7:

$$CP = (0.526 \times C16) - 4.992 \quad (7)$$

All these equations have been previously used to estimate the quality of algal biodiesel in comparison to biodiesel from different vegetable oils. In previous study^{37,40}, the accuracy of those empirical equations has been tested in reference to vegetable and microalgae oils, proving that the molecular structure of fatty acids directly affects the quality of the produced biodiesel.

Statistical analysis: Differences in different biochemical contents and pigments among lakes and seasons were tested separately by one-way analysis ANOVA followed by Tukey *post-hoc* test and correlation matrix using SPSS v20 package. Similarity analysis between lakes based on biodiesel quality criteria and fatty acids profiles were occurred by Primer v5.

RESULTS AND DISCUSSION

The maximum protein content (284.09 wwt%) found in summer in Aghormy Lake, while the minimum value of 189.39 wwt% was in autumn in Aghormy Lake with no significant differences between lakes or seasons. The average values of total protein content at the three lakes were maximum in summer, followed by winter and autumn. This contrariety with De Oliveira *et al.*⁴⁵, who mentioned that high water temperature, has been related to decrease the crude protein content. The maximum carbohydrate content was in Maraqi Lake in summer, reaching to 88.24 wwt%, while the minimum value was 33.17 wwt% in the same lake in winter with no significant differences. This agree with Abd El-Hady and Hussian⁴⁶ who found that the maximum level of total carbohydrate was in summer, while the minimum level detected in autumn in Ismailia Canal, River Nile. Carbohydrates and protein serve as storage components and the major form of photo-chemically assimilated carbon in the biosphere and are powerful tools in the pathways of biologically important organic materials in nature⁴⁷ (Table 1).

Algal lipids are composed of glycerol, bases of esterified saturated or unsaturated fatty acids. The total lipid content

Table 1: Pigments and biochemical contents of cyanobacterial mat

Parameters	Autumn			Winter			Summer		
	Aghormy	Zieton	Maraqi	Aghormy	Zieton	Maraqi	Aghormy	Zieton	Maraqi
Chlorophyll a ($\mu\text{g g}^{-1}$ wwt)	300.65	700.74	700.67	300.50	800.20	1100.50	500.72	700.44	1000.92
β -Carotenes (wwt%)	85.47	192.31	90.50	149.57	239.09	608.97	146.52	269.23	341.88
Phycocyanin (mg g^{-1} wwt)	1.85	1.08	1.84	4.99	2.55	2.85	3.50	2.65	4.60
Protein (wwt%)	189.39	197.63	218.53	222.33	202.92	216.45	284.09	195.90	218.53
Lipid (wwt%)	10.53	7.87	5.07	23.46	12.93	12.80	16.00	10.66	12.14
Carbohydrates (wwt%)	52.23	40.15	38.02	51.71	43.06	33.17	52.05	40.86	88.24
Glycerol (wwt%)	0.33	0.78	0.41	0.44	0.85	0.39	0.52	0.59	0.42
Ash (%)	25.80	38.01	12.62	27.14	13.25	16.03	19.34	18.30	19.08
Water contents (%)	46.58	56.78	44.69	63.44	36.99	36.97	44.97	43.76	44.41

reached maximum in winter in Aghormy Lake, with a value of 23.46 wwt%, while the minimum of 5.07 wwt% was detected in autumn in Maraqi Lake. The ratio carbohydrates/lipids were used as indicator of the chemical quality of the plankton⁴⁸. The present study shows that protein constitutes the major part of the biochemical contents of the cyanobacterial mat while lipid constitutes the minor one, this agrees with Renaud *et al.*⁴⁹ and Abd El-Hady and Hussian⁴⁶.

The maximum glycerol content (0.85 ww%) was recorded in winter in Zieton Lake, while the minimum value was 0.33 ww% in autumn in Aghormy Lake. ANOVA with *Post hoc* analysis indicated that there is a significant variation ($p < 0.01$) between lakes with a clear increase in Maraqi Lake (*Post hoc*, 0.56). The present data indicated that there is a relationship between the carbohydrate and glycerol contents; the carbohydrate synthesized during photosynthesis was consumed for glycerol synthesis, this was clear in Zieton Lake. These agree with Chitlaru and Pick²⁷, who reported that the decrease in carbohydrate content compensates the effect of accumulated glycerol on cell density. Also, Taha *et al.*⁵⁰ indicated that glycerol synthesis suppressed carbohydrate by nearly 4-11 times.

Along with chlorophyll a, the phycobiliproteins represent the major photosynthetic accessory pigments in cyanobacteria. There are three basic types of biliproteins; phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). The present investigation studied the phycocyanin (PC). The maximum PC content recorded in winter in Aghormy Lake while the minimum found in autumn in Zieton Lake. Thamizh and Sivakumar² reported that the level of PC was more than PE and APC and the chlorophyll a content is least. The natural detected levels of PC in Aghormy and Maraqi, 4.99 and 4.6 wwt% in winter and summer, respectively, were similar (4.8 dry wt%) to that reported by Leema *et al.*⁵¹ but lower than (12.6 dwt%) that reported by Chen *et al.*⁵². The detected PC in these studies was in optimized productions dry mass, whereas the results of our study were in naturally produced wet mats that mirrored the elevated levels of PC in Siwa lakes if

compared with the results obtained from the studies of Leema *et al.*⁵¹ and Chen *et al.*⁵².

β -carotene was economically important metabolic compounds. The maximum β -carotene was detected in winter in Maraqi Lake with the value of 608.97 wwt%, while the minimum value of 85.47 wwt% was found in autumn in Aghormy Lake. Karnjanawipagul *et al.*⁵³ found that the amounts of β -carotene in carrot was 6.19-14.59 wwt% which was similar to those (1.8-14.7 wwt% carrot) reported by Herrero-Martinez *et al.*⁵⁴. The present data indicated that the amount of β -carotene in cyanobacteria mat was 41 times more than in carrot. The highest amount of chlorophyll a was detected in Maraqi during winter, with a maximum of 1100.5 $\mu\text{g g}^{-1}$ wwt%. ANOVA with *Post hoc* analysis indicated that there is a significant variation ($p < 0.01$) between lakes with a clear increase in Maraqi lake (*Post hoc*, 736.8).

The correlation statistical analysis illustrated that, the total protein showed positive correlation with total lipid ($r = 0.54$) and with phycocyanin contents ($r = 0.39$). Also the phycocyanin content showed positive correlation with total carbohydrates and lipid contents ($r = 0.62$ and $r = 0.82$, respectively). Also chlorophyll a showed positive correlation with β -carotene content ($r = 0.81$).

The present data detected 34 fatty acids in the cyanobacterial mats at three sites of work in winter (Table 2). The lipid biomarker profiles reveal a surprising diversity of organic matter sources in the cyanobacterial mats of Siwa oasis. This agree with Rontani and Volkman⁵⁵ who detected that the marine microalgae are a major source of extractable lipids and a contribution to the fatty acids is also discernable. Maraqi contributed to the highest total fatty acids (1463.22 $\mu\text{g g}^{-1}$ dry wt.).

In the Aghormy Lake, the cis-10-heptadecenoic acid methyl ester (C17) is present in high concentration (456.14 $\mu\text{g g}^{-1}$ dry wt.) followed by palmitic acid methyl ester (C16) which present in significant amounts (208.02 $\mu\text{g g}^{-1}$ dry wt.). The cis-10-heptadecenoic acid (C17) content was on average, 43.03% of lipid content, while palmitic (C16), stearic

Table 2: Fatty acids ($\mu\text{g g}^{-1}$ dry wt.) of cyanobacterial mats in Siwa lakes

Fatty acids	Names	Aghormy	Maraqi	Zieton
C6:0	Caproic acid	nd	nd	2.21
C8:0	Caprylic acid	22.92	22.74	22.68
C10:0	Capric acid	28.12	32.86	35.25
C11:0	Undecanoic acid	6.17	2.77	2.77
C12:0	Lauric acid	7.80	8.28	14.99
C13:0	Tridecanoic acid	3.83	3.98	3.85
C14:1 ω 5	Myristoleic acid	11.77	19.65	8.24
C14:0	Myristic acid	18.46	28.24	39.41
C15:0	Pentadecanoic acid	7.74	10.94	13.16
C16:1 ω 5	Cis-10-pentadecenoic acid	9.59	10.31	10.80
C16:1 ω 7	Palmitoleic acid	14.33	74.21	47.81
C16:0	Palmitic acid	208.02	595.55	564.09
C17:1 ω 7	Cis-10-heptadecenoic acid	456.14	59.07	33.66
C17:0	Heptadecanoic acid	10.62	12.91	16.39
C18:3 ω 6	γ -Linolenic acid	16.70	17.01	16.96
C18:3 ω 3	Linolenic acid	16.74	18.25	17.21
C18:1 ω 9	Oleic acid	30.66	175.53	118.75
C18:1 ω 9	Elaidic acid	10.59	11.15	10.35
C18:0	Stearic acid	78.32	181.26	179.29
C20:4 ω 6	Arachidonic acid	19.17	16.80	nd
C20:5 ω 3	Cis-5,8,11,14,17-eicosapentaenoic acid	15.83	19.41	19.70
C20:3 ω 6	Cis-8,11,14-eicosatrienoic acid	17.03	16.89	16.90
C20:2 ω 6	Cis-11,14-eicosadienoic acid	15.07	15.22	15.20
C20:1 ω 9	Cis-11-eicosenoic acid	13.68	13.17	12.42
C20:3 ω 3	Cis-11,14,17-eicosatrienoic acid	15.23	15.24	15.25
C20:0	Arachidic acid	9.54	10.53	10.11
C21:0	Heneicosanoic acid	13.97	13.47	11.66
C22:6 ω 3	Cis-4,7,10,13,16,19-docosahexaenoic acid	24.42	19.21	18.51
C22:2 ω 6	Cis-13,16-docosadienoic acid	17.48	17.23	18.04
C22:0	Behenoic acid	23.08	20.34	20.31
C22:2 ω 6	Cis-13,16-docosadienoic acid	13.78	22.35	20.76
C24:1 ω 9	Nervonic acid	16.81	18.17	17.34
C24:0	Lignoceric acid	30.21	31.11	29.18
	Total fatty acids	1143.02	1463.22	1315.97

(C18) and oleic (C18) acid contents were in average; 19.63, 7.39 and 2.89% of the lipid content, respectively. Palmitic, stearic and oleic acids methyl ester were the most contributors to total fatty acids in Maraqi and Zieton. In Maraqi Lake (Table 2), the three acids had the values of 595.55, 181.26 and 175.53 ($\mu\text{g g}^{-1}$ dry wt.), respectively, with normalized dry lipid percentages of 58.39, 17.77 and 17.21%, respectively. In Zieton Lake, the three acid had the values of 564.09, 179.29 and 118.75 ($\mu\text{g g}^{-1}$ dry wt.), respectively, with normalized dry lipid percentages of 50.37, 16.01 and 10.6%, respectively.

The obtained data on fatty acids analysis are in agreement with previous findings for some of the studied strains⁵⁶. Nascimento *et al.*³⁸ found that palmitic acid (C16) was the predominant fatty acid in most of the algal lipid extracts. Knothe³⁵ conducted that palmitoleic (C16:1) acid is the most desirable to highly enrich in a fatty acid profile in order to overall improve biodiesel fuel properties. For comparison purposes, the fatty acid profiles of biodiesel (methyl esters) derived from two common commodity oils may be noted here. The fatty acids profile of soybean oil consists of

palmitic (usually 10-11%), stearic (4-6%), oleic (21-25%), linoleic (50-55%) and linolenic (=8%) acids, that of palm oil consists of palmitic (40-45%), stearic (4-5%), oleic (40%) and linoleic (10%) with lesser amounts of other fatty acids⁵⁷. Cyanobacterial fatty acids are mainly represented by C16 and C18 species⁵⁸. Some species, however, may have predominant C14 and C16 saturated and monounsaturated fatty acids⁵⁹.

The most important properties of the biodiesel potentially produced by the mats were estimated in this study (Table 3). This estimation allowed a comprehensive assessment of biodiesel quality according to the properties specifications. It has been reported that C16:1 and C18:1 fatty acids (palmitoleic acid and oleic acid, respectively) are most favorable for biodiesel production^{38,60}, these two acids had great importance especially in Maraqi Lake. Based on the results of this study, Zieton had the highest CN (57.2) and SV (184.34) whereas Aghormy had the highest IV (133.81) and DU (90.65), as well as best CFPP (-9.68) and CP (-3.55).

The ANP resolution⁶¹ specifies a minimum of 45 for CN. In the present study, all the studied mats showed CN values in a

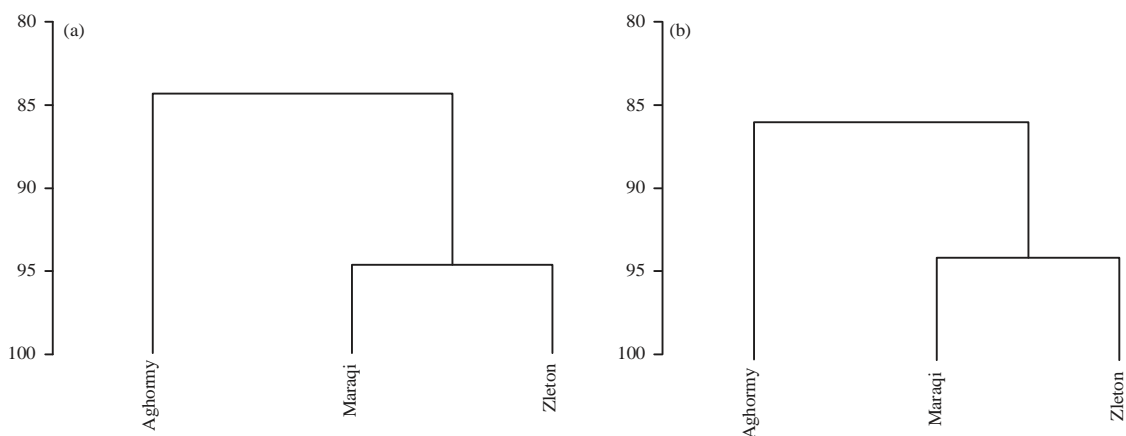


Fig. 2(a-b): Cluster analysis results comparing the variation of Siwa lakes based on (a) fatty acids profile and (b) biodiesel quality criteria

Table 3: Estimated properties of biodiesel from Siwa lakes

Siwa Lakes	CN	SV	IV	DU	LCSF	CFPP	CP	SFA	MUFA	PUFA
Aghormy	49.00	166.36	133.81	90.65	2.17	-9.68	-3.55	468.8	563.57	171.45
Maraqqi	45.12	221.20	114.93	73.65	3.46	-5.61	-0.72	974.98	381.26	177.61
Zieton	57.2	184.34	83.06	57.64	3.06	-6.86	-1.28	965.35	259.37	158.53

CV: Cetane number, SV: Saponification value, DU: Degree of unsaturation, LCSF: Long-chain saturated factor, CFPP: Cold Filter Plugging Point

similar range (between 45.12 and 57.2) as most of the biodiesels yield from algal cultures (40-65) as reported by Nascimento *et al.*³⁸ and (43.77-62.33) as reported by Talebi *et al.*⁴⁴ and for vegetable oils, such as 49 for sunflower, 52.9 for rapeseed and 50.9 for soybean⁶². The IV value of the oil originated from the algal mats is higher than that originated from algal culture investigated by Nascimento *et al.*³⁸, but was in the range of the investigation of Talebi *et al.*⁴⁴. The IV is a parameter not included in ASTM or Brazilian standards, even though it represents the DU, involving the weighted sum of the masses of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), important for the biodiesel oxidative stability. High unsaturation levels may result in polymerization of glycerides and formation of deposits⁶³. In comparison to biodiesel from vegetable oils⁶⁴, the estimated IV for the biodiesels from the algal mats (Table 3) was as equal as for soybean oil (120-141) and sunflower oil (110-143) except for the yielded oil from mats of Zieton Lake (83.06).

The CFPP values obtained by the oil of the three algal mats ranged between -5.61 and -9.68 that agree with⁶³, the CFPP obtained for microalgae oils varied from -12.3-20.8 °C. To achieve a balance between CN and CFPP, a biodiesel feedstock should have as high as MUFA content as possible¹⁷. The MUFA is generally the least abundant class of FAs in microalgae and so poor fuel properties are not surprising¹⁷.

This study indicated that Aghormy had highest MUFA value (563.57 $\mu\text{g g}^{-1}$ dry wt.) over PUFA and SFA that make the lake achieve the prospective balance between CN (49) and CFPP (-9.68). The increase of MUFA in Aghormy over Maraqqi and Zieton lakes was mainly due to the increase of cis-10-heptadecenoic acid (456.14, 59.07 and 33.66 $\mu\text{g g}^{-1}$ dry wt, respectively) and decrease of palmitic acid in Aghormy compared with Maraqqi and Zieton (208.02, 595.55 and 564.09 $\mu\text{g g}^{-1}$ dry wt., respectively). When Siwa lakes were compared based on fatty acids profile, saturation and unsaturation levels (Fig. 2a), as well as biodiesel quality criteria (Fig. 2b), two groups were identified by cluster analysis. Aghormy Lake was separated from Maraqqi and Zieton group, mirrored the specialized constituents of Aghormy oil (Fig. 2).

CONCLUSION

Generally, the highest biochemical contents, glycerol and pigments were detected in summer and winter. According to the present data, cyanobacteria mat is a good source especially for protein, phycocyanin and β -carotene contents. The detected amount of β -carotene in cyanobacteria mat was 41 times more than in carrot. The natural detected levels of PC in Aghormy and Maraqqi, 4.99 and 4.6 wwt% in winter and summer, respectively, were similar to that reported in

optimized dry mass production. The fatty acids profile revealed a surprising diversity in the Siwa oasis cyanobacterial mats included palmitic acid (C16), Cis-10-Heptadecenoic acid (C17), oleic and stearic acids (C18). The best biodiesel quality criteria were detected in Aghormy Lake. Nevertheless, the unhappy values observed for lipid content could be ascribed to the fact that this study did not intend to optimize lipid production, but in fact, was to screening the different biochemical contents as well as evaluating the significance of FA on biodiesel quality criteria. The cyanobacterial mats of Siwa oasis is considered as a renewable source for the production of pharmacologically, industrially important products and liquid fuels. As the algal mat highly tolerant to hyper saline water, making them promising for biodiesel production using seawater. Further research needs to be carried out in terms of technological feasibility on large scales and optimization in their natural occurrences.

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