UNLOCKING BIODIESEL POTENTIAL: PROFILING FATTY ACIDS FROM MICROALGAE ISOLATED FROM AGRICULTURAL SOIL

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Abstract. This study investigates the potential of using three microalgae as biodiesel substitutes. These microalgae were previously isolated from agricultural soil from Saudi Arabia. The isolates exhibited varying fatty acid profiles, with saturated fatty acids constituting nearly 50% of the total fatty acid composition and the polyunsaturated fatty acids constituting 37%-49%. Highly unsaturated fatty acids with more than four double bonds are absent in the investigated microalgae. In terms of biodiesel properties, all isolates demonstrated similar cetane numbers within the recommended standards. *Coelastrella sp.* UJ and *Chlorella sorokiniana* UJ had slightly elevated iodine values, but close to the recommended values. *Chlamydomonas zebra* UJ showed the lowest degree of saturation (DU=93), while *Chlorella sorokiniana* UJ had the highest (DU=108). The long-chain saturated fatty acids were highest in *Chlorella sorokiniana* UJ and lowest in *Coelastrella sp* UJ. Cold filter plugging point was around -16°C, slightly lower than the recommended values. Viscosity levels for all strains fell between 0.69 and 1.96, aligning with US minimum requirements but below EN standards. This research evaluated the viability of these microalgae as sustainable biodiesel sources. Based on these findings, future studies are necessary to optimize growth conditions, enhance fatty acid profiles, and maximize biodiesel yield.
Keywords: *algal biotechnology, algal fatty acids, sustainability, soil derived microalgae, biofuel, renewable energy*

Introduction

Microalgae constitute a wide diverse group of unicellular photosynthetic organisms converting light, carbon dioxide and water to algal biomass, which can be used in various renewable applications (Maltsev et al., 2020; Yap et al., 2021; Ng and Chew, 2024). They are distributed in almost all environments with a wide diversity in their morphological, physiological and biochemical characteristics (Gaurav et al., 2024). Microalgae have great potential as a rich and untapped resource for a wide range of applications in industrial and environmental sectors, such as human food, animal and aquaculture feed, and pharmaceuticals (Yap et al., 2021; Selvam et al., 2024).

Fatty acids are one of the major constituents of microalgal. They mainly exist in the form of glycerolipids, which are, in turn, the main constituents of the storage lipids—triacylglycerol (TAG)—and the membrane lipids, including phospholipids and other glycolipids (Maltsev and Maltseva, 2021). Microalgae TAG is considered as an important source of fatty acids of commercial interest. These fatty acids could be used as a resource for biofuel, nutraceuticals (ω-3 fatty acids) or in food commodities (Yap et al., 2021; Selvam et al., 2024). The total content of fatty acids in microalga as well as the length and degree of saturation of fatty acids are very variable among microalgae species. The major type of fatty acids produced by microalgae are fatty acids with chain lengths of 16 and 18 carbons (Maltsev and Maltseva, 2021). However, there are some other species which could synthesize longer fatty acids of up to 24 carbon atoms in length. Microalgae

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can produce both saturated, unsaturated fatty acids and highly unsaturated fatty acids. Unsaturated fatty acids produced by microalgae are of nutritional benefits. For example, the highly unsaturated ω-3 fatty acids; C20:5 (eicosapentaenoic acid; EPA) and C22:6 (docosahexaenoic acid; DHA) are important to keep heathy diet and have anti-inflammatory effects, they are produced by microalgae and there are no vegetable oil alternative to them (Maltsev and Maltseva, 2021; Conde et al., 2021; Dubey et al., 2024). The properties and qualities of microalgae fatty acids to be used as biofuels and edible oils is determined by the distribution of fatty acid chain length and degree of saturation (Selvam et al., 2024; Bharti et al., 2024).

Several microalgae have been studied intensively to determine their good nutritional quality, fatty acids profile and composition (Ng and Chew, 2024; Gaurav et al., 2024; Bharti et al., 2024). Therefore, the aim of this study is to investigate and profile the fatty acid composition of microalgae isolated from agricultural soil with the objective of evaluating their potential as a sustainable source of biodiesel.

Materials and methods

Microalgae isolates

Three microalgae isolates were obtained from agricultural soil at different locations in Saudi Arabia, as shown in the *Fig. 1*. Briefly, soil samples were collected in clean, sterilized plastic bags. The samples were then passed through a 2 mm sieve to remove larger particles before processing. Subsequently, the samples were exposed to light and enriched with BG-11 medium, allowing them to grow for 7 to 14 days. These isolates were identified by Alshareef (2021) and defined as *Chlamydomonas zebra* UJ, *Coelastrella sp.* UJ, and *Chlorella sorokiniana* UJ.

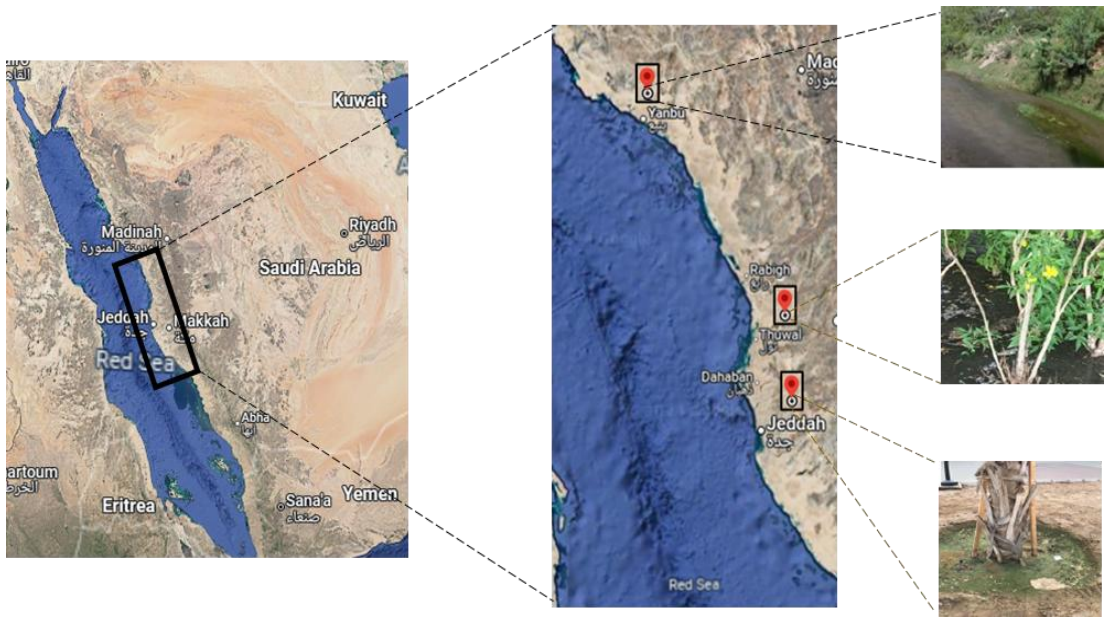

Figure 1. *Sampling sites in the western region of Saudi Arabia, highlighting the surrounding types of habitats*

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Microalgae growth

Microalgae isolates were cultivated in large aerated flasks containing BG-11 medium according to the method described in Rippka et al. (1979). The cultures incubated under controlled condition (temperature maintained at 22 ± 1°C, exposed to continues LED fluorescent light tube with intensity of 2000 LUX 28 μmol.m^{-2}. s^{-1}. The experimental set up is illustrated in *Fig. 2*.

Figure 2. *Experimental set-up used for cultivating microalgae*

Sample preparation, lipids extraction and transesterification

Crude microalgae were water rinsed to remove salt, lyophilized and analyzed for identification and quantification of individual fatty acids after hydrolyzing the lipids and converting each fatty acid to fatty acid methyl ester (FAME) analogues (Cavonius et al., 2014). Lipids extraction coupled with direct transesterification was performed according to Lewis method with slight modification (Lewis et al., 2000). Briefly, about 40 mg of freeze dried (lyophilized) microalgae were incubated at 90 °C for 120 min with methanol:HCl:chloroform (10:1:1). Then, 1.0 mL water (LC/MS grade) was added and the FAMEs extracted by adding 2.0 mL hexane–chloroform (4:1). After centrifuging for 5 min at 7500 rpm, aliquot of 170 μL of top layer was mixed with 30 μL of internal standard stock solution. Exactly 1 μL was injected into GC inlet using Gerstel autosampler.

Fatty acid profiling using GC/MS

The identification of microalgae FAMEs was performed by comparing retention time and MS spectra of individual FAME chromatographically separated from the sample to a standard mixture of 37 FAMES compounds.

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GC was carried out using Agilent 7890B GC instrument connected to EI triple quadruple mass spectrometer 7010B (Agilent technologies, USA). The chromatographic separation of the FAME was performed using capillary column HP-88; 30m long, 250 μm Id, and 0.2 μm film thickness (catalogue # 112-8867, Agilent technologies, USA). The final samples solution were analyzed by directly injecting 1 μL into multi-mode inlet (MMI) operated in split mode of 15:1. The following GC settings were used; initial oven temperature was 175 °C, ramped at a rate of 3 °C min^{-1} to 220 °C with 10 min hold time, the total run time was 35 min; injection temperature 280 °C; auxiliary temperature 320 °C; helium quench gas was set at 4 $mLmin^{-1}$ and nitrogen collision gas flow rate was 1.5 $mLmin^{-1}$. Helium (carrier gas) flow-rate was 1.0 mL min^{-1}. The triple quadrupole was auto tuned according to manufacturer tune manual with scan rate was set from 35 to 700 Da and dwell time of 100 msec. Identification of separated compounds was performed by NIST 14L MS spectra database library. The integration of the chromatographic peaks and quantification was performed using MassHunter Version B08.00 qualification and quantification software (Agilent technologies, USA).

Biodiesel properties

The biodiesel characteristics including cetane number (CN), long chain saturated factor (LCSF), saponification value (SV), cold filter plugging point (CFPP), iodine value (IV), and degree of unsaturation (DU) were using the formulas described (Ramos et al., 2009; Francisco et al., 2010) according to the equations from (1-6):

$$\mathrm{DU} = \mathrm{MUFA} + (2\mathrm{xPUFA}) \quad \text{(Eq.1)}$$

$$\mathrm{LCSF} = (0.1\mathrm{xC16{:}0}) + (0.5\mathrm{xC18{:}0}) + (1\mathrm{xC20{:}0}) + (1.5\mathrm{xC22{:}0}) + (2\mathrm{xC24{:}0}) \quad \text{(Eq.2)}$$

$$\mathrm{CFPP} = (3.1417\mathrm{xLCSF}) - 16.477 \quad \text{(Eq.3)}$$

$$\mathrm{CN} = 46.3 + \left(\frac{5458}{\mathrm{SV}}\right) - (0.255\mathrm{xIV}) \quad \text{(Eq.4)}$$

$$\mathrm{SV} = \sum \frac{560\mathrm{F}}{\mathrm{Mw}} \quad \text{(Eq.5)}$$

$$\mathrm{IV} = \sum \frac{245\mathrm{FD}}{\mathrm{Mw}} \quad \text{(Eq.6)}$$

where,
F: represents proportion of each fatty acid (as % of total fatty acids),
D: represents number of double bonds,
Mw: represents molecular weight of fatty acid.

Statistical analysis

All experiments were done in two technical replicates and data were presented as mean ± SE and analyzed using Graph Pad Prism 10.2.0 and Microsoft Excel 2019. The significance of differences was assessed using ANOVA test, considering values of $P < 0.05$ as significant.

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Results

Fatty acid content and distribution

Chlamydomonas zebra UJ and *Chlorella sorokiniana* UJ have a similar content of fatty acids while *Coelastrella sp.* UJ has higher content (*Fig. 3a*). About 44% of the total fatty acids in *Chlamydomonas zebra* UJ was saturated fatty acids (SFAs) while *Coelastrella sp*. UJ and *Chlorella sorokiniana* UJ has a slightly less SFAs content (39% to 41%, respectively). The content of monounsaturated fatty acids (MUSFAs) is between 9% to 19% of the total fatty acids with *Chlorella sorokiniana* UJ has the lowest MUSFAs (9%). *Chlamydomonas zebra* UJ has the lowest content of PUFAs and *Chlorella sorokiniana* UJ has the highest as almost half (49%) of the total fatty acids content is from PUFAs (*Fig. 3b*).

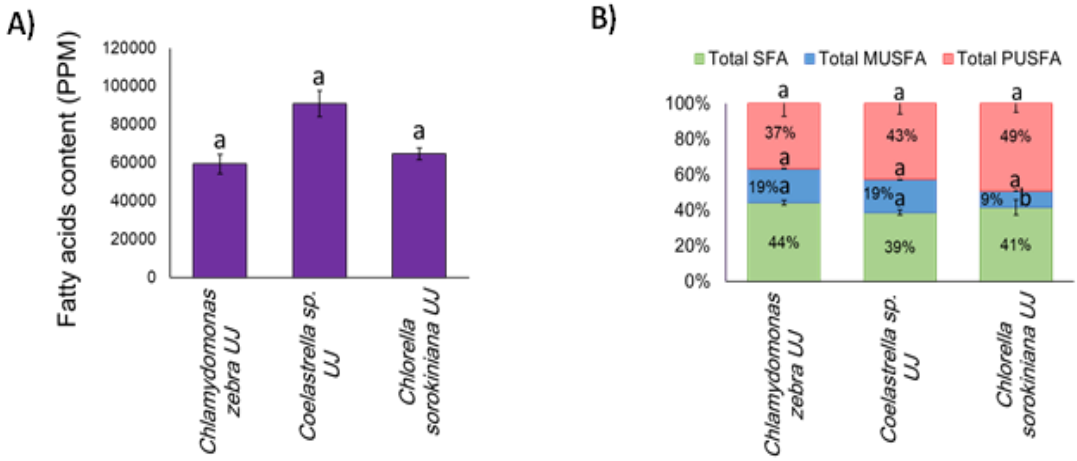

Figure 3. *Fatty acid content of microalgae isolates. (A) Total fatty acids content calculated as ppm. (B) Distribution of each class of fatty acids*

Fatty acids composition

Based on the FAs profiling, majority of SFAs (79% to 83%) in microalgae isolates are from Palmitic acid (C16:0). Stearic acid (C18:0) is the second most abundant SFAs which constitutes 11-13% of the total SFAs. Arachidonic acid (C20:0) is present in a very little amount in *Chlorella sorokiniana* UJ (*Fig. 4a*). We also reported other SFAs of shorter chain length such as (C14:0) but with very little amount (2-3%) and other fatty acids with odd number of carbons such as (C15:0) and (C17:0) with little amounts which also constitutes 2-3% of the SFAs.

For MUFAs, oleic acid (C18:1) constitutes 57% and 72% of the total MUFAs in *Chlorella sorokiniana* UJ and *Coelastrella sp.* UJ respectively, while it is absent in *Chlamydomonas zebra* UJ which has two C18:1 isomers identified in the mass spectroscopy spectra. Regarding C16:1, we have identified two isomers constituting 28% to 43% in all three microalgae (*Fig. 4b*).

Regarding PUFAs, Linoleic acid (LA, C18:2) and alpha linolenic acid (ALA, C18:3) constitutes the highest PUFAs available in all three isolated microalgae with *Chlamydomonas zebra* UJ having similar content of LA (C18:2) and ALA (C18:3) which together constitutes 68% presents in a similar ratio (*Fig. 4c). Coelastrella sp.* UJ has about 41% of its PUFAs as LA (C18:2) and ALA (C18:3). While *Chlorella sorokiniana* UJ has

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both LA (C18:2) and ALA (C18:3) which present in 9:1 ratio of LA to ALA. Other unknown C16:2, C16:3, C16:4, C18:3 and C18:4 is also identified in the microalgae investigated in this research. C16:2 present in 9% to 21% in all three isolates, while C16:3 present in 8% to 23% in all three isolates. *Coelastrella sp.*UJ and *Chlorella sorokiniana* UJ has about 15% of their PUFAs as C16:4 while *Chlamydomonas zebra* UJ has no C16:4. Regarding C18:3 and C18:4, all three microalgae have C18:3 present in 5% to 16%, while C18:4 present in very little amounts from 6% to 8% in *Chlamydomonas zebra* UJ and *Coelastrella sp.*UJ, respectively. *Chlorella sorokiniana* UJ has no C18:4 fatty acids (*Fig. 4d*).

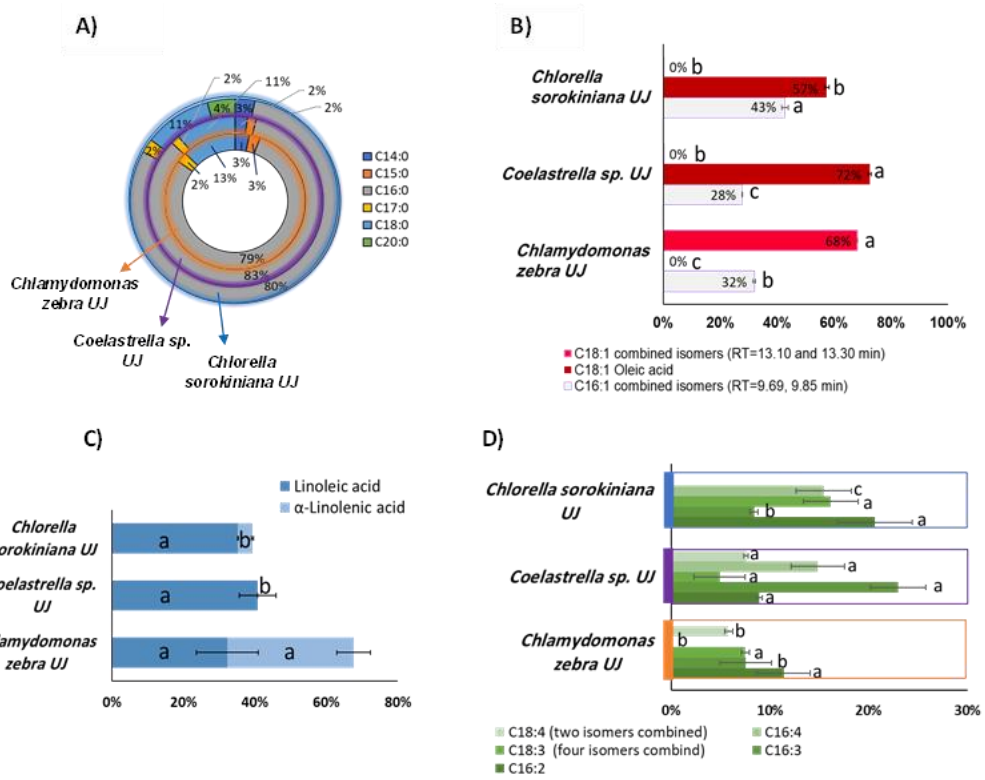

Figure 4. *Distribution of different classes of fatty acids. (A) Percentage of different saturated fatty acids relative to the total saturated fatty acids. (B) Percentage of monounsaturated fatty acids relative to the total monounsaturated fatty acids. (C) Percentage of linoleic acid and a-linolenic acid to the total polyunsaturated fatty acids. (D) Distribution of other unknown polyunsaturated fatty acids to the total polyunsaturated fatty acids*

Analysis of biodiesel properties

To evaluate the effectiveness of the microalgae isolates to be used as a potential substitute of diesel, several parameters including cetane number (CN), iodine value (IV), saponification value (SV), degree of saturation (DU), Long Chain Saturated Factor (LCSF), cold filter plugging point (CFPP), viscosity (υ), density (ρ) and linolenic acid methyl ester content have been calculated. In the current research, the CN value of all microalgae isolates ranges between 42 to 46 (*Table 1*), this value is close to the acceptable range of European and US standards (Which is ≥ 47 in ASTM and ≥ 51 in EN). Regarding the IV, two microalgae isolates; *Coelastrella sp.* UJ and *Chlorella sorokiniana* UJ have IV value slightly higher (IV= 123 and 125 g/100 g, respectively) than EN standard limit

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(maximum limit of 120 g Iodine/100 g fat) (*Table 1*). With respect of SV, all strains explored in this research have similar SV. All of microalgae evaluated in this study has at least 50% of their FAMEs as SFAs and MUFAs. Interestingly, most of the PUFAs are from two or three double bonds FAs, and very little amounts of four double bonds FAs (their four double-bonds PUFAs not exceeding 9% of the total FAs, with *Chlamydomonas zebra* UJ having only minor concentration ~2% of the total FAs). The lowest DU was noted in *Chlamydomonas zebra* UJ (DU= 93) and the highest DU was reported in *Chlorella sorokiniana* UJ (DU=108) (*Table 1*). The high DU value in *Chlorella sorokiniana* UJ is attributed to its high content of PUFAs (49% of its total FAs compared to 37% in *Chlamydomonas zebra* UJ).

Table 1. *Biodiesel properties of selected microalgae compared with the standard values of the American Society for Testing and Materials (ASTM) and the standard as neat biodiesel (B100) (Hoekman et al., 2012; Islam et al., 2013)*

Biodiesel Property	***Chlamydomonas zebra***	***Coelastrella sp.***	***Chlorella sorokiniana***	**ASTM D6751-02**	**EN 14214**
Cetane number (CN)	42 ± 3 [a]	46 ± 1 [a]	45 ± 7 [a]	≥ 47	≥ 51
Iodine value (IV) (g Iodine/100g fat)	136 ± 10 [a]	123 ± 7 [a]	125 ± 50 [a]	-	≤ 120
Saponification value (SV) (mg KOH/g)	204 ± 60 [a]	201 ± 5 [a]	202 ± 80 [a]	-	-
Degree of saturation (DU)	93 ± 5 [a]	104 ± 7 [a]	108 ± 3 [a]	-	-
Long Chain Saturated Factor (LCSF)	6.3 ± 1 [a]	5.4 ± 0.7 [a]	7.32 ± 0.1 [a]	-	-
a-Linolenic acid methyl ester content C18:3	2.6 ± 0.5 [b]	2.10± 0.1 [b]	10 ± 0.98 [a]	-	≤12
Kinematic viscosity (υ) at 40 °C (mm^2/s)	0.69-1.72	0.88-1.72	0.69-1.95	1.9-6.0	3.5-5.0
Density (ρ) (g/cm^3)	0.86-0.9	0.86-0.9	0.86-0.9	NA	0.86-0.9
Cold Filter Plugging Point CFPP (ºC)	-16.29 ± 05 [a]	-16.32 ± 3 [a]	-16.25 ± 1.8 [a]	-	-5 to -13

In addition to the high DU in *Chlorella sorokiniana* UJ, it also has the highest LCSFs value (LCSFs value= 7.32) (*Table 1*) due to the presence of C20:0 which is absent in the other microalgae investigated in this study. *Coelastrella sp.* UJ has the lowest (LCSF=5.4) due to its decreased content of C16:0 and C18:0 compared to *Chlamydomonas zebra* UJ and the DU value less than *Chlorella sorokiniana* UJ (*Table 1*). With respect to the presences of C16-C18 FAs, our data showed that the *Chlamydomonas zebra* UJ has high content of C16 and C18 species (78% of total FA content), while the strains *Chlorella sorokiniana* UJ and *Coelastrella sp.* UJ have a moderate content at 62%-67%, respectively.

The alpha linolenic acid methyl ester for all studied strains is less than 10% with *Chlamydomonas zebra* UJ and *Coelastrella sp.* UJ having only 2% and *Chlorella sorokiniana* UJ having 10%. These levels are in-line with EN standard which recommend linolenic acid methyl ester level less than 12% (*Table 1*).

Regarding the density and viscosity of the biodiesel, viscosity for all strains ranged between 0.69 to 1.96 which is less than the range of EN (3.5-5). However, the viscosity

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level close to the minimum requirement of US standards which is between 1.9-6.0. All strains have density level similar to EN standards (0.86-0.90 g/cm^3).

The CFPP values of the biodiesel from the strains studied in the present research was around -16 °C (*Table 1*) for all strains, this value is within and even less than the EN standard (CFPP between -5 to -13). We reported low presence of palmitic (below 35%) in all strains, with *Coelastrella sp.* UJ having the lowest percentage (32%), while the maximum stearic acid was 6% (in *Chlamydomonas zebra* UJ*)*, and low steric acid (4% to 6%) in the strains studied in this research (*Table 2*).

Table 2. *Palmitic and steric acid content of selected microalgae. Fatty acid percentage was calculated as relative to total FAs*

	Chlamydomonas zebra		***Coelastrella sp.***		***Chlorella sorokiniana***	
	FA content PPM	**FA %**	**FA content PPM**	**FA %**	**FA content PPM**	**FA %**
Palmitic acid (C16:0)	20725.2 ± 19 ^{b}	35 % ± 0% ^{b}	29274.5 ± 890 ^{a}	32 % ± 1% ^{a}	21489.1 ± 1700^b	33 % ± 3% ^{b}
Steric acid (C18:0)	3311.8 ± 650 ^{a}	6 % ± 1% ^{a}	3963.9 ± 189 ^{a}	4 % ± 0% ^{a}	2826.1 ± 189 ^{a}	4 % ± 0% ^{a}

Discussion

The increasing demand for sustainable energy sources granted significant interest to microalgae as alternative source for biodiesel production, due to their high lipid content and their robust growth. Microalgae are able to accumulate high levels of lipids which can be converted into biodiesel via transesterification. Fatty acids profiles can vary significantly among different microalgae species. In our study, we found palmitic acid (C16:0) is the major SFAs followed by stearic acid (C18:0) and then arachidonic acid (C20:0) which is the least abundant, this is consistence with other microalgae species which has palmitic acid and steric acids as the most abundant SFAs (Suastes-Rivas et al., 2020; Arutselvan et al., 2021; Pereira et al., 2021). We also noted the presence of other short chain FAs such as (C14:0) that present in minimal amount (2-3%) and other odd number FAs such as (C15:0) and (C17:0), these FAs may indicate contamination of microalgae culture with bacteria and/or cyanobacteria (Grubišić et al., 2022). However, it has been reported that some microalgae have also these types of FAs but, as a rule, their content should not exceed 3–5% of all FAs. *Scenedesmus obliquus* and *Dunaliella salina* are two examples, which has 5.0% of their FAs as C14:0 (Moreno-Garcia et al., 2021). Nevertheless, there are some microalgae strains which can have up to 15% of their total FAs as C14:0. For example, *Chlamydomonas asymmetrica* synthesizes C14:0 in an amount of 14.2%, *Chlorella sp.* in an amount of 10.9% (Lang et al., 2011; Wang et al., 2021; Krivina et al., 2024) and *Rhizoclonium riparium* in an amount of 8.05% (Osuna-Ruiz et al., 2019).

Previous research showed that the LA content in microalgae is highly variable. For example, the content of LA in *Heterococcus endolithicus* is 53.37% (Lang et al., 2011) and in *Bracteacoccus bullatus* is 23.8% (Maltsev et al., 2020) while in *Bracteacoccus bullatus* is 13.9% of LA (Mamaeva et al., 2018). Here we found that the LA content in the *Chlorella sorokiniana* UJ studied in this research is similar to the ratio reported by which is 36.0% (Alsenani et al., 2020). Previous reports reported that green microalgae exhibit a significant concentration of ALA. For instance, certain *Chlamydomonas* strains have been found to contain up to 62.3% ALA (Lang et al., 2011). Similarly, *Dunaliella*

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tertiolecta has been reported to possess 60.2% ALA (Nielsen et al., 2019), while *Chaetopeltis orbicularis* can contain as much as 57.5% ALA (Lang et al., 2011; Chen et al., 2021). Additionally, *Carteria* can have an ALA content of up to 54.6% (Lang et al., 2011), and *Scenedesmus obliquus* has been observed to contain 41.17% ALA (Oliveira et al., 2020). The *Chlamydomonas* studied in this research showed a different-very little-ALA -compared to the *Chlamydomonas* reported in Lang et al. (2011) study.

To evaluate the effectiveness of the microalgae isolates to be used as a potential substitute of diesel, several parameters including cetane number (CN), iodine value (IV), saponification value (SV), degree of saturation (DU), Long Chain Saturated Factor (LCSF), cold filter plugging point (CFPP), viscosity (υ), density (ρ) and linolenic acid methyl ester content have been described (Lim et al., 2023; Elsharaihy et al., 2024; Balouch et al., 2023). High CN value of biodiesel is indicative of superior combustion, reduced nitrous oxide (N_2O) emissions, decreased instances of engine knocking, and easier engine start-up (Arguelles and Martinez-Goss, 2021). Previous reports specify a minimum CN value of ideal biodiesel of 47 (CN=47) (Manzoor et al., 2022). Here we found the CN value of all microalgae isolates close to the acceptable range of European and US standards. The variation in CN values can be attributed to different strains being evaluated. Iodine value is another important characteristic in determining the susceptibility of biodiesel to polymerize. From our study, the two microalgae isolates; *Coelastrella sp.* UJ and *Chlorella sorokiniana* UJ have IV value slightly higher than EN standard limit. Elevated IV can lead to the polymerization of glycerides and thus accumulation of lubricant deposits within the engine (Farfan-Cabrera et al., 2022).

Achieving a low degree of unsaturation (DU) and good level of long chain saturated fatty acids (LCSFs) are fundamental in optimizing the overall functionality and performance of diesel engines, as high content of PUFA decrease the stability of biodiesel (Manzoor et al., 2022). In this study, all evaluated microalgae exhibited at least half of their FAMEs as SFAs and MUFAs. This characteristic is advantageous, as it suggests that these microalgal strains possess the necessary lipid profiles for producing stable biodiesel. Moreover, the presence of a high proportion of SFAs and MUFAs not only contributes to improving the oxidative stability but also enhancing the overall fuel quality, making these microalgae good candidates for biodiesel production. Previous studies (Atmanli, 2020; Yang et al., 2023) described feedstocks suitable for biodiesel production to be enriched with five common fatty acids with chain length of C16-C18. These include C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), and C18:3 (linolenic acid) (Dębowski et al., 2021). Our data showed that the *Chlamydomonas zebra* UJ has high content of C16 and C18 species, while the strains *Chlorella sorokiniana* UJ and *Coelastrella sp.* UJ have a moderate content (62%-67%).

Saturated fatty acids generally have higher melting points compared to unsaturated fatty acids. When the oil predominantly consists of saturated fatty acid, there is a possibility of crystallization taking place within the normal operating temperature range of the engine (Bharti et al., 2024). This can give rise to unfavorable Cold Filter Plugging Point (CFPP) properties, negatively impacting the efficiency of the system. Hence, biodiesel containing a high proportion of palmitic and stearic acid methyl esters is prone to exhibiting inferior CFPP characteristics, resulting in a higher temperature at which plugging occurs (Enwereuzoh et al., 2020). The CFPP values of the biodiesel from the strains studied in the present research was within and even less than the EN standard (CFPP between -5 to -13) although other previous studies showed higher CFPP values of oil obtained from microalgal (range between −12.3 to 20.8 °C) (Enwereuzoh et al., 2020).

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Conclusion

Currently, screening and evaluating the lipid profiles and fuel properties of potential microalgae are crucial for determining the feasibility of utilizing algal oils as a substitute for biodiesel. The fatty acid profile significantly influences the main characteristics of biodiesel. This work investigated and described the fatty acid profiles of three microalgae native to the environment of Saudi Arabia. *Chlamydomonas zebra* UJ and *Coelastrella sp.* UJ exhibited favorable biodiesel characteristics, such as low degree of unsaturation (DU), a moderate content of long-chain saturated fatty acids (LCSF), and low content of alpha-linolenic acid (ALA, C18:3); however, they showed slightly higher iodine value (IV) and saponification value (SV). Nevertheless, these characteristics make these two isolates good candidates for further assessment for biodiesel production. Future studies are needed to optimize growth conditions to enhance fatty acids profiles and biodiesel yield.

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