



Biodiesel Production By *Chara Vulgaris* Isolated From Freshwater Of Basrah Province, Iraq

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Abstract

The negative impacts of burning fossil fuels on the environment and the rising crude oil costs have increased the interests in biofuel. The only renewable biofuel that is able to replace fuels made from petroleum is biodiesel produced from algae. The current study was conducted to assess the efficiency of green macroalgae, *Chara vulgaris* and the possibility of using it as a source of alternative energy production. Algae samples were collected from the freshwater environment in Basrah city. Algae were subjected to phenotypic diagnosis, then genetically identified based on ITS1 amplification. The sequences of the gene were identified and matched with the database in GenBank. The algae were identified as *Chara vulgaris* with an identity of 100%. The oil was extracted from algal biomass in two ways, at room temperature and in the soxhlet extraction device, yielding 0.09 and 0.163% oil, respectively. The oil esterification process was carried out using two types of catalysts, basic and acidic catalysts. The result of the esterification process was analysed by GC/MS, showing that fatty acid esters were the highest, while fatty acids and hydrocarbons were low. An assessment of the physical properties of the biodiesel produced was also carried out, proving to be non-carbon 0%, and of low sulphur and water content. These characteristics were compared to those of oil diesel.

Keywords: Biodiesel, *Chara vulgaris*, Macroalgae, Biofuel.

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Introduction

The growth in the consumption of energy based on fossil fuels has led to many environmental issues related to global warming, climate change and continuous emission of greenhouse gases, such as carbon dioxide (Appiah-Nkansah *et al.*, 2019). Moreover, this type of non-renewable energy is rapidly depleting and oil reserves and expected to disappear in 2050 (Ma *et al.*, 2019). Therefore, the search for economic, renewable and environmentally friendly sources of energy is the primary goal of this century (Yang and Yang, 2019). The use of biofuels based on biomass will not only mitigate global warming, but also reduce carbon dioxide emissions due to the prior consumption of this gas by biomass through the photosynthesis process to emit neutral carbon (Amoah *et al.*, 2019).

Biodiesel, one of the alternatives to fossil fuels, is produced from various raw materials, including oils extracted from crops (Ma *et al.*, 2019), animal fats and microorganisms (Nguyen *et al.*, 2020). Biodiesel is a liquid fuel chemically composed of alkyl esters of fatty acids, which are produced from the reaction of triglycerides or fatty acids derived from vegetable oils, animal fats, microalgae and macroalgae with short-chain alcohol in the presence of a stimulator (Kumar *et al.*, 2020).

Algae have received attention as a new source of biomass for producing renewable energy compared to other biomass sources due to their availability around the world, rapid growth and high biomass productivity (Saad *et al.*, 2019). They also can adapt to any surrounding environment and grow in wastewater that contains many nutrients that promote the growth of macroalgae (Rajhi *et*

al., 2020). In addition, they play a vital role in the global carbon cycle, significantly impacting the bio-fixation of carbon dioxide; macroalgae are the main product that forms food chains in fresh and marine waters (Bhuyar *et al.*, 2021). *Chara vulgaris* is one of the large algae found in local ponds and swamps. The research focused on extracting oil from it, and demonstrating the efficiency of oil in biodiesel production using acid and trans esterification.

Material and methods

Collection and preparation of raw material

Macroalga was collected from Basrah city for the period from January 2019 to February 2020 and October 2020 to February 2021. The pre-treatment process was carried out by cleaning the samples several times with tap water, and washing them with distilled water, drying them in shade for 72 hours, stirring and grinding using an electric mill for 2 min to obtain a fine powder, and then keeping them in containers until further use (Nageswara and Al Riyami, 2018).

Genetic Identification

The DNA was extracted using Genomic DNA Mini Kit (Plant) according to the manufacturer's protocol. For the polymerase chain reaction amplification and sequencing of ITS1-5, the PCR thermo cycling profile included an initial denature step at 94°C for 5 min; followed by 30 cycles of denaturation at 94°C for 30 sec, primer annealing at 57°C for 30 sec and elongation step at 72°C for 1 min; and a final elongation at 72°C for 5 min. Polymerase chain reaction amplification of ITS region were carried out by using the primers listed in Table 1.

Table 1, Sequence of primers used to amplify the ITS1 region

| Primer | Sequence | Reference |
|--------|-----------------------------|---------------------------------|
| ITS1 F | 5"-TACCTGGTTGATCCTGCCAG-3" | (Nakayama <i>et al.</i> , 1996) |
| ITS1 R | 5"-TAACTAAGAACGGCCATGCAC-3" | (Hoshina <i>et al.</i> , 2005) |

Biochemical composition

Total carbohydrates

The carbohydrates were extracted from the biomass by acid hydrolysis. Briefly, 0.1 g of dry algae powder was taken and digested with

5 ml of 2N sulfuric acid and placed in an autoclave at 120°C for 30 min (Miranda *et al.*, 2012). Then, the mixture was neutralized with solid sodium carbonate, Na₂CO₃, diluted and filtered in the centrifuge (Bhagea *et al.*, 2022).

The total carbohydrates were determined by phenol-sulphuric acid method (Dubois *et al.*, 1956).

Total proteins

The protein was estimated using the micro-kjeldahl method (AACC, 2000) and percentage of protein was calculated by using a 6.25 conversion factor.

Total lipids

The method by (Subramanian *et al.*, 2015) was used with some modifications: 20g dry algae powder was placed in a thimble with 250 ml of solvent mixture chloroform: methanol (2:1 v/v) in continuous extraction. The mixture was filtered with filtration papers and the mixture was evaporated to 100 ml, and then transferred to a separation funnel before washing with 25 ml of the aqueous solution of sodium chloride, NaCl (0.85%). The organic layer was collected. The solvent was disposed of algae oil and weighed to determine the amount of oil produced by the biomass.

Cold extraction method

The method by (Kumar *et al.*, 2011) was used with some modifications: 10 g of algae powder was mixed with 100 ml of solvent mixture chloroform: methanol (2:1 v/v) under constant stirring using a magnetic stirrer for four hours. After that, the mixture was filtered using a filter paper. Re-extraction with 25 ml of chloroform twice was done to extract the remaining oil in the dry powder. Then, it was transferred to separation funnel, after washing with 25 ml of NaCl aqueous solution (0.85%), the organic layer was collected and the solvent was evaporated to obtain algal oil that was weighed to determine the amount of oil produced from the biomass.

Effect of alcohol volume

Algal oil was mixed with methanol containing 2 ml of sulphuric acid. The same previous steps were repeated, replacing the catalyst with 0.3 g potassium hydroxide (KOH). Methanol was used in three different volumes (40, 60 and 80 ml) for both treatments with acid and base catalyst (El-Shimi *et al.*, 2013). The transesterification was performed using

the basal catalyst, 0.3 KOH (w/size), at 55-60°C for two hours (Nageswara and Al Riyami, 2018). The esterification was conducted using 2 ml of H₂SO₄ (v/size) and methanol at 55-60°C for 5-6 hours (El-Shimi *et al.*, 2013).

Transesterification

Methanol was mixed with 0.3 g KOH (w/v) under constant stirring using a magnetic stirrer to dissolve KOH and to form methoxide. Methoxide was added to the extracted oil, and placed in water bath to maintain the interaction heat (55-60°C). We used reflux condenser to reduce the loss of solvent. After 90 minutes, the mixture was left for one hour to settle. Then, the mixture was filtered using filter paper and washed twice with 10 ml of methanol for 10 minutes to remove any traces of remaining fatty acids (FA). The resulting liquid was added to the previous mixture and 15 ml of distilled water was added to facilitate the separation. The mixture was separated by centrifuge at 5000 rpm for 15 min. The mixture was transferred to a separation funnel. Then, 10 ml of hexane was added to the mixture and shook well. The hexane layer was taken. This process was repeated several times. The hexane layer was washed with distilled water to remove the residues of catalyst and methanol, dried and kept until the physical and chemical qualities were estimated (Kim *et al.*, 2014).

Esterification

Methanol was mixed with 2 ml of sulfuric acid (vol/vol) under constant stirring using a magnetic stirrer. Then, it was added to the extracted oil and placed in water bath to maintain the interaction heat (55-60°C). We used reflux condenser to reduce the loss of solvent. After 5h minutes, the mixture was left for one hour to settle. Then, the mixture was filtered using filter paper and washed twice with 10 ml of methanol for 10 minutes to remove any traces of the remaining fatty acids (FA). The resulting liquid was added to the previous mixture and 15 ml of distilled water was added to facilitate the separation. The mixture was separated by centrifuge at 5000 rpm for 15 min. The mixture was transferred to a separation funnel. Then, 10 ml of hexane

was added to the mixture and shook well. The hexane layer was taken. This process was repeated several times. The hexane layer was washed with distilled water to remove the residues of catalyst and methanol, dried and kept until the physical and chemical qualities were estimated (Kim *et al.*, 2014). The analysis of fatty acid was conducted in the Department of Research and Quality Control, Nahr Bin Omar Basrah Oil Company in Basrah, Iraq, by using Gas Chromatography.

Estimation of physical qualities

The density, kinematic viscosity, pour point, sulphur content, underdeveloped carbon content, water content and diesel index were evaluated in Laboratories and Quality Control

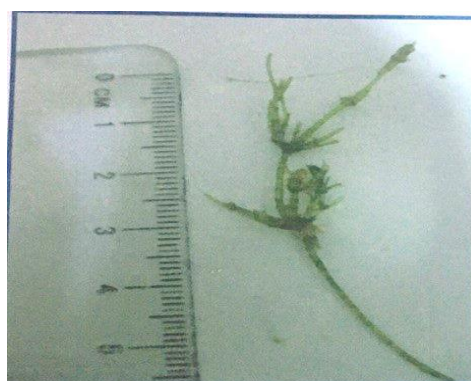


Fig 1, Morphology of *Chara vulgaris*

Depending of the genetic identification, the results showed that the algae isolates were identified according to the ITS1 amplification. Based on the BLAST results, the algae belonged to *Chara vulgaris* species with 99% identity and Sequence ID: [HF913645.2](#).

Biochemical content

We noted the variation in the proportions of chemicals found in *Chara vulgaris*. Carbohydrates were the highest, with a percentage of 72%, followed by proteins (6%) and oil (3.19%) Table 2.

Table 2, Chemical composition of *Chara vulgaris*

| No | Compounds | Percentage |
|----|--------------|------------|
| 1 | Protein | 6.023 |
| 2 | Carbohydrate | 72.59 |
| 3 | Lipid | 3.19 |

Department Quality Management system, Basrah Oil Company.

Statically analysis

Statistical analysis was performed using SPSS software (V24) two way test.

Results

Chara vulgaris macroalgae attached to rock and plant, with their distinguished root and shoot system comprising of well-developed vegetative and reproductive structures. The body consisted of a long, cylinder, and jointed green main axis with regular successions of node and internode. The central axis was branched, and each node gave rise to a whorl of lateral branches as shown in Fig 1.

The oil ratios for both types of extraction are shown in Table 3. The hot extraction was the highest, with a percentage of 0.163%.

Table 3, Extraction of oil from algae biomass

| Type of extraction | Oil g/ 20 gm. |
|--------------------|---------------|
| Cold extraction | 0.09 |
| Hot extraction | 0.163 |

The amount of produced esters is shown in the Table 4, noticed the higher amount in acid catalyst for both types of extraction, with the highest percentage of ester extracted using 80 ml of the acidic catalyst (15.48 g/kg), while the lowest percentage was 80 ml of the basic catalyst, amounting to 1.02 g/kg.

Table 4, Amount of fatty acid methyl ester (FAME g/kg) yield from *Chara vulgaris*

| Hot extraction | | | | | | Cold extraction | | | | | |
|-----------------|------|-------|----------------|------|------|-----------------|------|------|----------------|------|------|
| Acidic catalyst | | | Basic catalyst | | | Acidic catalyst | | | Basic catalyst | | |
| 40ml | 60ml | 80ml | 40ml | 60ml | 80ml | 40ml | 60ml | 80ml | 40ml | 60ml | 80ml |
| 3.52 | 5.57 | 15.49 | 1.85 | 1.17 | 1.02 | 8.15 | 7.94 | 4.23 | 1.97 | 2.42 | 2.65 |

The physicochemical properties of biodiesel are shown in Table 5, was observed that the density ratios were similar for all treatments, as well as the viscosity, except for 60 ml which was the lowest among them 0.53, the

percentages of sulphur and water content were low, except for 40 ml of an acid catalyst, with 0.37 and 0.08% respectively, and no residual carbon content was recorded.

Table 5, Physicochemical properties of biodiesel extraction from *Chara vulgaris*

| Properties | Method | Acid cold 80 ml | Acid hot 40 ml | Base cold 60 ml | Diesel |
|--------------------------------------|-------------------|-----------------|----------------|-----------------|--------|
| Density (g/cm ³) @15.6 c | ASTM D-4052 | 0.6646 | 0.6643 | 0.6645 | 0.87 |
| Kinematic viscosity @40 c | ASTM D-445 | 1.528 | 1.821 | 0.537 | 12-18 |
| Pour point | ASTM D-97 | Bellow -30 | Bellow -30 | Bellow -30 | +9 |
| Sulphur content | ASTM D-4294 | 0.0216 | 0.3704 | 0.0141 | 2.5 |
| Carbon residue, wt% | ASTM D-4530 IP-13 | NIL | NIL | NIL | 1.5 |
| Water content by Karl Fisher, vol. % | ASTM D-6304 | 0.01 | 0.08 | 0.01 | 0.5 |

Table 6, shows the percentage of hydrocarbons in cold extraction. Tetracosane was the highest percentage 3.5744%, followed by octadecane 1.724% the highest percentage

of total hydrocarbon was in the 60 ml MeOH treatment, yielding 7.05%, and the lowest was in 40 ml 1.68%.

Table 6, Hydrocarbon compounds for cold extraction (acid catalyst)

| Compounds | 40ml MeOH | 60ml MeOH | 80ml MeOH |
|------------------------------------|------------|-----------|-----------|
| | Percentage | | |
| Undecane | 0.0133 | 0.0275 | 0 |
| Dodecane, 1,1-dimethoxy- | 0.5058 | 0 | 0 |
| Octadecane, 1-(ethenyloxy)- | 1.1502 | 0 | 0 |
| Octadecane | 1.6616 | 1.724 | 0 |
| Nonacosane | 0.9132 | 0 | 0 |
| Undecane, 2,6-dimethyl- | 0 | 0.0975 | 0.0980 |
| Dodecane, 4,6-dimethyl- | 0 | 0.1083 | - |
| Cyclotetradecane | 0 | 0.0724 | 0.0578 |
| Hexadecane, 2,6,10,14-tetramethyl- | 0 | 0.3937 | 0 |
| Tetracosane | 0 | 3.5744 | 0 |
| 1-Nonadecene | 0 | 0.4043 | 0 |
| 1-Hexacosene | 0 | 0.6562 | 0 |
| 1,19-Eicosadinene | 0 | 0 | 0.0295 |
| 7-Tetradecene, (E)- | 0 | 0 | 0.0168 |
| Dodecane, 2,6,10-trimethyl- | 0.0926 | 0 | 0.2668 |
| Docosane | 0 | 0 | 0.9677 |
| Total | 4.3367 | 7.0583 | 1.6875 |

In the hot extraction, tetracosane was the highest 1.40%, but its percentage was lower than the cold extraction of the same catalyst,

followed by heneicosane 1.075%, the highest percentage of total hydrocarbons was in the treatment of 60 ml, yielding 4.702% Table 7.

Table 7, Hydrocarbon compounds for hot extraction (acid catalyst)

| Compounds | 80ml MeOH | 60ml MeOH | 40ml MeOH |
|----------------------------|------------|-----------|-----------|
| | Percentage | | |
| 1-Docosene | 0.8999 | - | - |
| Heneicosane | 1.0756 | - | - |
| Undecane | - | 0.0837 | - |
| Dodecane, 4,6-dimethyl- | - | 0.0239 | - |
| Hexacosane | - | 0.0324 | - |
| Tridecane | - | 0.0251 | - |
| Cyclohexadecane | - | 0.3828 | - |
| Eicosane | - | 0.6528 | - |
| Octadecane, 1,1-dimethoxy- | - | 0.8161 | - |
| Tricosane | - | 0.7672 | - |
| Tetracosane | - | 1.4081 | - |
| 1-Hexacosene | - | 0.3028 | - |
| Docosane | - | 0.1818 | - |
| Nonane2,-methyl | - | 0.0253 | - |
| Total | 1.9755 | 4.702 | 0 |

The results for using the basic catalyst, Z-14-nonacosane appeared as the highest percentage 4.1432% among all compounds, followed by tetracosane 2.2889% as shown in

Table 8, the highest percentage of total hydrocarbons was in the treatment of 40 ml, reaching 16.4373%.

Table 8, Hydrocarbon compounds for cold extraction (basic catalyst)

| Compounds | 80ml MeOH | 60ml MeOH | 40ml MeOH |
|---|------------|-----------|-----------|
| | Percentage | | |
| Undecane | 0.0366 | - | - |
| Octadecane | 0.1336 | - | 0.2611 |
| Cyclododecane | 0.0924 | - | - |
| Nonacosane | 0.3258 | 0.3309 | 0.1446 |
| Hexadecane | 0.0659 | 0.0768 | 0.1908 |
| Cyclotetradecane | - | 0.0303 | - |
| Tetracosane | 0.8376 | 0.8433 | 2.2889 |
| 1-Nonadecene | - | - | 0.1189 |
| 1-Hexacosene | 0.4959 | - | 0.5784 |
| Docosane | 0.1276 | 0.859 | 0.7074 |
| 3-Heptadecene, (Z)- | 0.1397 | - | 0.6617 |
| 3,5-Dimethyldodecane | 0.4568 | - | - |
| 2-Methyl-Z-4-tetradecene | 0.0629 | - | - |
| Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)- | 0.1313 | 0.1437 | - |
| 5-Methyl-Z-5-docosene | 0.1151 | 0.2322 | 0.0884 |
| Nonadecane | 0.2862 | - | - |
| Hexacosane | 0.1325 | 0.6741 | 0.8691 |
| Tricosane | 1.4232 | - | 2.8786 |
| Pentacosane | 0.6589 | - | 0.1978 |
| Dodecane, 2,6,11-trimethyl- | - | 0.4358 | - |
| Tetradecane | - | 0.0271 | 0.0419 |
| 3-Eicosene, (E)- | - | 2.5794 | - |

| | | | |
|--|--------|--------|---------|
| Pentadecane | - | - | 0.3389 |
| Cyclohexadecane | - | 0.0248 | - |
| 2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane | - | 0.062 | - |
| Heptadecane, 2,6,10,14-tetramethyl- | - | - | 0.0437 |
| 7-Hexadecene, (Z)- | - | - | 0.1143 |
| Heptadecane | - | - | 0.6248 |
| Heneicosane | - | - | 0.2745 |
| 5-Tetradecene, (E)- | - | - | 0.0457 |
| 1-Octadecene | - | - | 0.156 |
| Eicosane | - | - | 0.9194 |
| Heptadecane, 2-methyl- | - | - | 0.0601 |
| Heptadecane, 3-methyl- | - | - | 0.0384 |
| Octacosane | - | - | 0.1732 |
| 1,9-Tetradecadiene | - | - | 0.2222 |
| Cyclopentadecane | - | - | 0.1505 |
| Z-14-nonacosane | 4.1432 | 0.0536 | 3.9972 |
| Hentriacontane | - | - | 0.0752 |
| Hexadecane,3-methyl | - | - | 0.1756 |
| Total | 9.6652 | 6.373 | 16.4373 |

For the hot extraction, only one compound, nonadecane 0.3683%, appeared in the 80 mL MeOH, and it did not appear in the other two treatments. The results of the statistical analysis showed no significant difference ($P \leq 0.05$), but the standard deviation in the case of hydrocarbons 60 ml was the best. As for fatty

acids, no difference was observed between them. In general, the polyunsaturated fatty acids were of a low percentage, and 9,12-Octadecadienoic acid (Z,Z)- had the highest rate of 1.0339% and gamolenic acid was the least 0.2135% Table 9.

Table 9, Fatty acid for cold extraction

| Compound | Carbon numbers | Acid catalyse | | | Base catalyse | | |
|----------------------------------|----------------|---------------|-----------|-----------|---------------|-----------|-----------|
| | | 80ml MeOH | 60ml MeOH | 40ml MeOH | 80ml MeOH | 60ml MeOH | 40ml MeOH |
| | | Percentage | | | Percentage | | |
| Linoelaidic acid | C18:2 | 0.6673 | - | - | - | - | - |
| 9,12-Octadecadienoic acid (Z,Z)- | C18:2 | - | - | 1.0339 | - | - | - |
| 9-Octadecenoic acid, (E)- | C18:1 | - | - | - | 0.4355 | - | - |
| n-Hexadecanoic acid | C16:0 | - | - | - | - | - | 0.4683 |
| Gamolenic acid | C18:3 | - | - | - | - | - | 0.2135 |
| Total | | 0.6673 | 0 | 1.0339 | - | - | 0.6818 |
| Σ SFA | | - | - | - | - | - | 0.6818 |
| Σ MUSFA | | - | - | - | 0.4355 | - | - |
| Σ PUSFA | 0 | 0.6673 | 0 | 1.0339 | 0 | 0 | 0.2135 |

SFA: saturated fatty acid

MUSFA: monounsaturated fatty acid

PUSFA: polyunsaturated fatty acid

The results in Table 10 , showed the fatty acids of hot extraction. The saturated fatty

acids were higher than the unsaturated fatty acids, and n-hexadecanoic acid was the

highest, with percentage of 31.6566% and eicosanoic acid was the least 0.1682%.

Table 10, Fatty acid components after hot extraction by acid and base

| Fatty acid | C No. | Acidic catalyst | | | Basic catalyst | | |
|--------------------------------------|----------|-----------------|-----------|-----------|----------------|-----------|-----------|
| | | 40ml MeOH | 60ml MeOH | 80ml MeOH | 40ml MeOH | 60ml MeOH | 80ml MeOH |
| | | Percentage | | | Percentage | | |
| 22-Tricosenoic acid | C23:0 | 1.8478 | - | - | - | - | - |
| Linoelaidic acid | C18:2 | - | 0.4277 | - | - | - | - |
| Dodecanoic acid | C12:0 | - | - | 0.2479 | - | - | - |
| Tetradecanoic acid | C14:0 | - | - | 1.2075 | - | - | - |
| Pentadecanoic acid | C15:0 | - | - | 0.6001 | - | - | - |
| Palmitoleic acid | C16:1n-7 | - | - | 0.502 | 0.286469 | - | - |
| n-Hexadecanoic acid | C16:0 | - | - | 31.6565 | - | - | - |
| Heptadecanoic acid | C17:0 | - | - | 0.9095 | - | - | - |
| cis-Vaccenic acid | C18:1n-7 | - | - | 1.9463 | - | - | - |
| Octadecanoic acid | C18:0 | - | - | 3.8069 | - | - | - |
| Eicosanoic acid | C20:0 | - | - | 0.1682 | - | - | - |
| Docosanoic acid | C22:0 | - | - | 0.4261 | - | - | - |
| Oleic Acid | C18:1 | - | - | - | 0.58521 | 0.30963 | 2.78721 |
| cis-10-Heptadecenoic acid | C17:1 | - | - | - | 4.774951 | 4.236053 | - |
| cis-13-Eicosenoic acid | C20:1n-7 | - | - | - | 1.234137 | 0.83113 | 0.68624 |
| Oxiraneoctanoic acid, 3-octyl-, cis- | C18:1 | - | - | - | - | 0.891458 | - |
| cis-13-Octadecenoic acid | C18:1n-5 | - | - | - | - | 0.584417 | - |
| Total | | 1.8478 | 0.4277 | 41.471 | 6.880767 | 6.852688 | - |
| ∑SFA* | | 1.8478 | - | 39.0227 | - | 0.58441 | - |
| ∑MUSFA* | | - | - | 2.4483 | 6.880767 | 6.268271 | 3.47345 |
| ∑PUSFA* | | - | 0.4277 | - | - | - | - |

*SFA: saturated fatty acid, MUSFA: monounsaturated fatty acid, PUSFA: polyunsaturated fatty acid

The standard deviation showed a high dispersion in the case of hydrocarbon fatty acid for both types of catalysts.

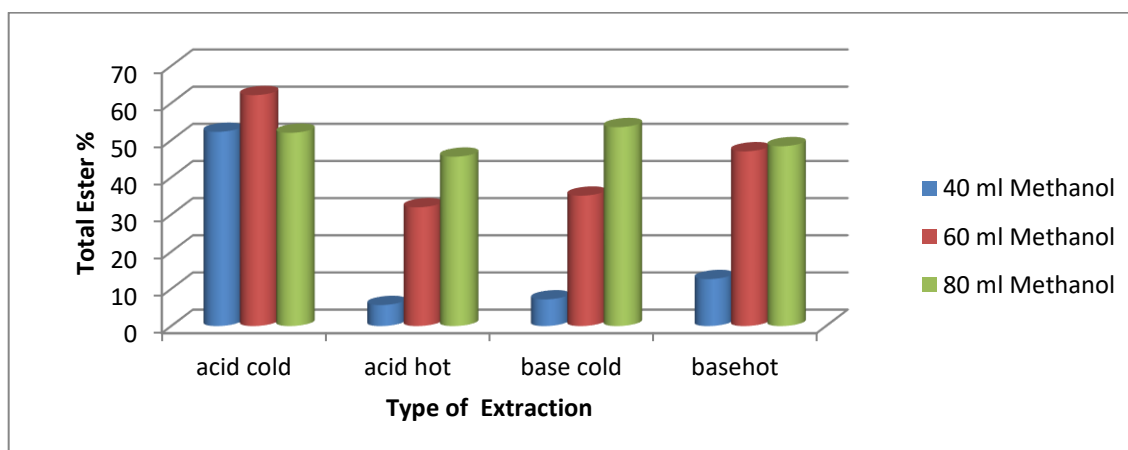


Fig 2, The ratio of total esters for hot and cold extraction

The results of the statistical analysis showed that there were no significant differences ($P < 0.05$) between the amount of alcohol used, but the results of the standard deviation showed that the concentration of 80 ml methanol was the best for obtaining the highest amount of ester, followed by the concentration of 60 ml methanol. The acid catalyst was better than basic catalyst. In the case of the efficiency of the extraction type, no significant differences were observed between them ($P < 0.05$).

Discussion

The results of the current study Table 2, showed a high percentage of carbohydrates in *Chara vulgaris*, amounting to 72.28%. The reason may be due to the influence of the climate, especially high temperatures throughout the year, according to (Meng and Srivastava 1993), photosynthetic activity has a positive effect on the accumulation of carbohydrates since there is a positive association between the increase in carbohydrates and day length and temperature (Margret *et al.* 2008; Tredici *et al.*, 1991) indicated that the carbohydrate content in biomass was higher on sunny days compared to cloudy days (Markou *et al.*, 2012), These conditions are available in the local environment as it is characterized by sunny days almost all year round. The low percentage of oil, which amounted to 3.19% might be attributed to nutrient deficiencies, making the algae to accumulate fat within its tissues, or to exposure of the algae to long periods of light. (Hotimchenko, 2002) indicated that macroalgae grown in shade contained a higher percentage of total fat compared to algae grown in full light (Gosch *et al.*, 2012) and the amount of low oil produced in this study was similar to the study by (Siddiqua *et al.* 2015), reaching 3.66%. It was also higher than the percentage of oil in a study by (Trifa *et al.*, 2013), which amounted to 1.06%. The reason may be due to the difference in the geographical location and environmental conditions for the growth of each algae, in addition to the different period of sampling. From observing the results of Table 2, we noted that the percentage of

proteins was 6.023%, the second highest ratio after carbohydrates.

The oil was extracted in this study Table 3, by two methods of extraction at room temperature on a stirrer device, and instant extraction in a Soxhlet device. The percentage of oil with hot extraction was higher than cold extraction by 0.164% and 0.09%, respectively, and this was consistent with the study by (Whangchai *et al.*, 2021), which reported 3.36% and 7.86% for cold and hot extraction, respectively, but differed from a study by (Saengsawang *et al.*, 2020), which had the highest percentage of oil using simple extraction amounting to 0.37%, followed by soxhlet extraction (0.16%). The high temperature increases the amount of oil produced, leading to an increase in biodiesel productivity (Jayakumar *et al.*, 2021). It was also noted that the amount of fatty acid esters decreased using the basic catalyst as indicated in the research by (Eze *et al.*, 2014), The high percentages of potassium hydroxide may cause a decrease in the ester yield due to the saponification processes that occur for fatty acid esters; this was what appeared in this study although the potassium hydroxide was less than 8.5%.

Physicochemical properties of fuel shown in Table 5, reported that the density was low for all treatments, which was lower than in the biodiesel standard between 0.87-0.89 g/ml. It was also lower than reported by (Siddiqua *et al.*, 2015) the density of *Chara* algae was 0.87 g/ml, the viscosity was less than 0.6 for one treatment only, and the other was between 1.5-1.8, which was low compared with the viscosity of diesel of 5 because of this low viscosity, it cannot be used as fuel directly, but it is either added or mixed with the fuel as observed in a study by (Rani and Kumar, 2021).

Table 6-7, and Table 8, showed that the concentration of hydrocarbons was low. The reason might be due to the effect of environmental conditions on algae, especially since the samples were collected during the winter when the temperature of air and water were low. This was also observed in the study

of (Hmeed *et al.*, 2014), The lowest concentration of hydrocarbon compounds for the studied algae was in the winter season and the highest concentration was in the spring as a result of the algae blooming and an increase in their metabolic activity. It was also noted in the current study that the concentration of hydrocarbons increased in cold extraction compared to hot extraction due to the fact that they are volatile compounds that volatilize at high temperature. Among these compounds are heptadecene and octadecene, which were also found in the study of (Rzama *et al.*, 2002) on the alga, *Chlorella vulgaris*, but in higher proportions than the current study. The study also found saturated hydrocarbons tetracosane and octacosane, and these compounds also were extracted from the alga, *Botryococcus braunii* at 17% and 14.8%, respectively (Dayananda *et al.* 2007). However, in the current study, their percentages were lower with 0.8376-2.288% and 0.1732%, respectively. The reason for the difference in hydrocarbon content may be due to the different strains to which it belongs, as well as the physiological and culture conditions (Dayananda *et al.*, 2005). We also found isomers of heptadecane, which were 3-heptadecane and 7-heptadecane. This type of compound is prevalent in red and brown algae, Heptadecane is the predominant compound in the hydrocarbon fraction of all microorganisms.

From the results of Table 9-10, the saturated fatty acids were higher than unsaturated fatty acids, with the highest being hexadecanoic acid (palmitic acid), which constituted between 40-35% of total fats. which is what was found in this study, as it was the highest with a percentage of 31.65% and that the high of palmitic acid. The observation could be due to higher temperatures in the environment, indicating that the increase in temperature stimulates the proportion of palmitic acid (Hu and Gao, 2006). This is in agreement with the study by (Krohn *et al.*, 2011) , which indicated that palmitic acid was one of the most common fatty acid derivatives among different types of algae (Asikainen *et al.*, 2015) , Likewise, the study by (Anwer *et al.* 2022) reported the highest percentage of

palmitic acid in *Chara* algae by 46.07%, as for C18 unsaturated fatty acids, they were present in low rates between 1.9-3.1%.

From the results, esters were the highest percentage, with significant differences between them and hydrocarbons and fatty acids. We noticed Fig 1, that the percentages were high in the hot extraction, and the fatty acid esters were the highest in the basic and acidic catalysts meanwhile, for the cold extraction, the acidic catalyst yielded the highest esters of saturated fatty acids; the ester of 9.12-octadecanoic acid methyl ester was the highest 19.21% this ester was also found in the study of (Sonawane *et al.*, 2015) , but at 8.12%, which was the lowest in the current study.

Conclusion

Algae is one of the most important organisms that can be used for sustainable energy production. They are autotrophs that can be harvested from the environment in large quantities and their sustainable development and tolerance to environmental fluctuations. The kara moss has been shown to contain a quantity of fats, which represent the raw material for the production of biodiesel, which is a form of clean energy that is environmentally friendly.

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