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Lipid extraction from some seaweeds and evaluation of its biodiesel production

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ABSTRACT

The present study evaluated the efficiency of seaweeds collected from Abu Qir Bay, Alexandria, Egypt, as a feedstock for biodiesel. A total of 15 macroalgal species were collected overall in the four seasons. The highest seasonal biomass production of 652.1 and 626.3 g m^{-2} was recorded with *Chaetomorpha linum* and *Ulva compressa* in autumn and spring, respectively. While the highest annual biomass production was detected in *Ulva fasciata* (1056.8 g m^{-2}). Lipid content varied among species, with the highest value of 14.66% and 9.94% dw in *U. fasciata and U. compressa* during spring, which resulted in the highest annual lipid productivity of 67.4 and 63.3 g m^{-2}, respectively. Palmitic acid (C16:0) showed the highest value among all fatty acids (1.12–19.62 mg g^{-1} dw) in all studied species. The biodiesel characteristics of all algae species tested are in agreement with the values of international standards. Overall, the present study recommended *U. compressa* and *U. fasciata as* a promising biodiesel feedstock due to the relatively higher lipid productivity and FAMEs characteristics that comply with the international standards and high net energy output that reached 1.24 and 1.30 GJ ton^{-1}, respectively.

1. Introduction

The world's population is rapidly increasing, and by 2050, it is predicted to exceed 9 billion people (Dutta et al., 2014). Due to greenhouse gas (GHG) emissions from uncontrolled fossil fuel consumption, this rapid increase resulted in energy shortages and unfavorable environmental effects (Berardi, 2017). Fossil fuels have been consumed quickly, which led to a need to find an alternate fuel to achieve world demand. Therefore, fossil-based energy's green commutation is the trending approach that has gained much interest from research sectors and governments worldwide. Biodiesel, biobutanol, bioethanol, and biogas are examples of biomass-based fuels that are renewable, sustainable, and environmentally benign. When biodiesel is used in a traditional diesel engine, carbon monoxide, unburned hydrocarbons, and particulate matter are significantly reduced. (Murugan et al., 2019).

Biodiesel, a nonpetroleum-based fuel, described as long chain fatty acid mono-alkyl esters extracted from oils of vegetables or fats of animals with lower molecular weights alcohols, mainly methanol, in the existence of catalyst. Biodiesel as alternate diesel fuel has recently gained massive interest worldwide due to its sustainability, biodegradability, and good exhaust emission. The recent research of the use of substitute, non-food related feedstock such as algae oil is becoming common. Algae are capable of turning carbon dioxide into biomass, which can be further refined downstream to generate fertilizers, biodiesel, and other useful products (Sheehan et al., 1998). Furthermore, algae are considered to be one of the critically important future supplies of renewable biofuels (Abomohra et al., 2018; El-Shenody et al., 2019) and have been identified as promising sunlight-driven cell factories for the converting of carbon dioxide to biofuels and chemical feed stocks (Abomohra et al., 2019; Li et al., 2015).

Algae have several features that allow them to be a sustainable biodiesel feedstock that merits more study. Algae can grow and produce useful by-products such as carbohydrates, lipids, proteins, and several feedstocks that can be transformed into biofuels in addition to other beneficial materials. Although microalgae contain a high oil content, they are difficult to be cultivated and harvested cost-effectively. Differently, macroalgae or seaweeds offer low-cost cultivation and harvesting capacity (Sheehan et al., 1998). Many macroalgae species contain a considerable amount of lipid contents that make them promising candidates for biodiesel production (Abomohra et al., 2018; Elshobary et al., 2020a,b; Gosch et al., 2012). The seasonal variation of macroalgae's biochemical components determines the yields of

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biodiesel, bioethanol, and biogas (Gosch et al., 2012; Ismail et al., 2020).

The significance of macroalgae in bioenergy development is owing to marine algae need smaller land areas than conventional crops and do not need arable land for growth (Abomohra et al., 2016). In addition, they don't compete on freshwater and produce biomass at a faster rate (Abomohra et al., 2018; Subhadra and Edwards, 2011). Consequently, algal production yield per unit area in a certain time, known as productivity, is significantly larger than those for terrestrial biomass. In that context, the possible biological resources of marine environments at Rocky Bay of Abu Qir in Egypt have not been adequately investigated for bioenergy applications. Therefore, the present study aimed to assess the seasonal variation of macroalgae collected from Abu Qir Bay. The biochemical composition of the collected seaweeds was studied and evaluated for biodiesel production in biomass, lipids, and FAMEs. Furthermore, the biodiesel characteristics of the studied species were compared with those of the international standards, and the net energy output was calculated for the most promising species.

2. Materials and methods

2.1. Materials

Sulfuric acid, phenol, sodium hydroxide, phosphoric acid, Coomassie; 8 Brilliant Blue G-250, Bovine Serum Albumin, organic solvents, D-glucose, and other chemicals used in this study were obtained with a purity of 95–99 percent from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) or Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Seaweeds collection

Seaweeds were collected seasonally in October, January, April, and July (2017–2018) from Abu Qir Bay area, Alexandria, Egypt. The collected seaweeds were washed carefully with marine water, tap water and transferred to the laboratory in iced boxes. Identification was carried out morphologically according to (Aleem, 1993; Jha et al., 2009; Kanaan H. and Belous o, 2016) and using the Algae Base website (Guiry and Guiry, 2019). The collected samples were air-dried in the shade at room temperature for 3–5 days then in the oven at 38 ± 2 °C until constant weight. The dried seaweeds were ground into a fine powder and stored for further studies in tightly closed containers at −4 °C. Seasonal and annual biomass was determined for each species as gram cellular dry weight (CDW) per square meter (g m^{-2}).

2.3. Seawater physicochemical properties

Seawater was seasonally (October, January, April, and July) sampled from the site of the algal collection. The water surface temperature, pH, humidity, conductivity, salinity, and seawater turbidity were recorded *in situ.* The pH, humidity, and temperature were measured using a digital pH meter (Research model 201/Digital pH meter). Total dissolved solids and conductivity were measured by a conductivity meter (HANNA HI 98130). Turbidity was estimated by a mini turbidity meter (CRISON INSTRUMENTS, S.A.). The rest of the seawater parameters were measured in the lab, including ammonium (NH_4^+), calcium (Ca^{++}), magnesium (Mg^{++}), salinity, total hardness as $CaCO_3$, chloride (Cl^-), Sulfate (SO_4^-), bicarbonate (HCO_3^-), nitrate (NO_3^-), nitrite (NO_2^-) following the American Public Health Association's protocol of standard methods (APHA) (APHA, 1998) .

2.4. Biochemical composition of seaweeds (primary metabolites)

2.4.1. Estimation of total soluble carbohydrates and proteins

The seaweed sample (0.1 g powder) was extracted with NaOH (1 N) in a boiling water bath at 100 °C for 2 h, according to (Payne and Stewart, 1988). The extract was cooled, centrifuged, and kept for carbohydrates and proteins estimation. Content of total soluble carbohydrates was quantified by the method of Phenol-Sulfuric acid described by (Kochert, 1973). The absorbance was determined at 490 nm against a blank (0.5 ml of 1 N NaOH was used instead of seaweed extract). Total carbohydrate content was given as a percentage of the macroalgal dry weight (% dw) using D-glucose as a standard. The total content of soluble proteins was assessed using the method of (Bradford, 1976). Total protein content was given as a percentage of the seaweed dry weight % dw using Bovine Serum Albumin as a standard.

2.4.2. Estimation of lipids

2.4.2.1. Total lipid content. Lipid was extracted according to the modified Folch method (Folch et al., 1957) with some modifications. 3 g of powdered dried seaweed sample was mixed with 90 ml of chloroform/methanol (2/1, v/v). The mix was maintained on a rotatory shaker at 120 rpm for 48 h at room temperature. The homogenate was filtered using filter paper (Whatman No.1) to separate the liquid phase. The liquid phase was washed with an amount of 0.9% NaCl (w/v) to form two separating phases. The organic phase of lipid extract was moved to a preweighted glass vial and dried at 40–45 °C until constant weight. The quantity of total lipid content was expressed as % dw. Seasonal and annual productivity has been expressed as (g m^{-2}).

2.5. Fatty acid analysis

A- Preparation of methyl ester of fatty acids

The transmethylation of lipids and extraction of fatty acid methyl esters (FAMEs) were prepared, as mentioned by (Radwan, 1978).

B- Gas liquid chromatography of methyl esters of fatty acids and FAMEs characteristics

Gas chromatography–mass spectrum (GC-MS spectrophotometry PerkinElmer model: Clarus 580/560 S) was used to analyze the fatty acid methyl esters (FAMEs). GC-MS spectrophotometry equipped with Rxi- 5 Sil MS column (30 m × 0.25 mm). The oven temperature program started at 60 °C for 2 min, which raised by 10 °C/min to 280 °C and hold for 6 min. The detector and injector temperatures were adjusted at 250 °C and 200 °C, respectively. A sample volume of 1 μL of FAMEs dissolved in a constant amount of petroleum ether was injected at a split ratio of 20:1 using helium as carrier gas at a flow rate of 1 mL min^{-1}. Fatty acids methyl ester were estimated per dry weight using trinonadecanoylglycerol as an external standard for fatty acids. The estimated quality biodiesel product from the definition of fatty acids profile take place by calculation the main chemical and physical properties, including the average degree of unsaturation (ADU, %), kinematic viscosity (υi, mm^2 s^{-1}), specific gravity (SG, kg^{-1}), cloud point (CP, °C), cetane number (CN), iodine value (IV, g I_2 100 g^{-1} oil), and higher heating value (HHV, MJ kg^{-1}), according to (Hoekman et al., 2012) as previously described by (Elshobary et al., 2020a,b).

2.6. Statistical analysis

Analysis of variance (ANOVA) was used with a 0.05 level of significance to determine the degree of seasonal variation. Duncan's multiple comparison tests were used to compare mean values for seawater physicochemical properties and biochemical composition of the collected seaweeds using SPSS v.23.

3. Results and discussion

3.1. Water characteristics

Abu Qir Bay is one of the most ecologically sensitive coastal areas in

Egypt, located 35 km east of Alexandria. There are three holes (Rosetta mouth of Nile River, El-Tabia pumping station, and Lake Edku outlet) that supplies the Bay with different types of continental drainage. The seasonal variations in the environmental conditions and water composition at the collection site are listed in Table 1. The average surface seawater temperature (SST) in summer and autumn was a little higher (25 °C and 24 °C, respectively) compared to the spring and winter seasons. The highest humidity percentage was recorded in winter and spring of 30%, while autumn and summer showed the lowest percentages, 25%, and 20%, respectively. The pH values varied from 6.0 in spring and winter to 7.3 in autumn with no significant differences. There is no difference in water color among seasons. Autumn and spring showed the maximum turbidity value of 5.5 and 6.0 NTU, respectively, while winter and summer recorded only 4 NTU. The highest value of conductivity and total dissolved solids (TDS) was recorded in winter (65,000 μmhos cm^{-1} and 39,000 mg L^{-1}, respectively).

Concerning dissolved anions, chloride showed the highest value of 23,700 mg L^{-1} during winter, while the lowest value of 17,100 mg L^{-1} was recorded in autumn (Table 1). Sulfate showed it maximum content in autumn and winter (496 and 400 mg L^{-1}, respectively), compared to spring and summer of 100 and 20 mg L^{-1}, respectively. Bicarbonate HCO_3^- ranged between 904 mg L^{-1} in winter to the highest content of 6785 mg L^{-1} in autumn. Nitrate and nitrite showed the highest values of 4.3 and 0.28 mg L^{-1}, respectively, in autumn. However, dissolved cations such as ammonium, calcium, and magnesium showed the highest values of 26.5, 4656, and 2567.4 mg L^{-1} in autumn, winter, and autumn, respectively. Salinity recorded the highest value of 39,000 me L^{-1} in winter, while autumn showed the lowest value of 30,000 me L^{-1}. Nitrate and ammonium enrichment is due to the municipal wastewater effluent in this area. The algal community affected by the change of physical and chemical characterization of water during the different seasons (Elshobary et al., 2020a,b). and biomass production (Osman et al., 2020) as discussed in the following section.

3.2. Seaweeds and biomass production

A total of 15 macroalgal species, including 5 Chlorophytes, 2 Phaeophytes, and 8 Rhodophytes, were collected (Table 2). The seasonal and annual variations in the macroalgal biomass production are shown in Fig. 1. Season, population structure, and a number of other ecological factors all influence seaweed biomass and species composition. (Thakur et al., 2008). Results showed that the highest seasonal biomass production was recorded with *Chaetomorpha linum* and *Ulva compressa* of Chlorophyta in autumn and spring (652.1 and 626.3 g m^{-2}, respectively). However, most of the species' biomass production was relatively low in winter due to the environmental conditions and water composition at the collection site in this season, which is in agreement with (Kang et al., 2011), who compared the macroalgal growth in different seasons and concluded a decrease in algal growth in cold weather. Oh et al. (2016) demonstrated that temporal and spatial changes determine the seaweeds biomass where the seaweeds biomass decreased in winter seasons in two sites (Tonggae and Sinjeonri) while showed opposite results in Ando,

Table 2
Seaweeds species collected from Rocky Bay of Abu Qir in different seasons from October 2017 to July 2018.

Season	Division	Name of collected algae
October (2017) Autumn	Chlorophyta	*Chaetomorpha linum*
		Ulva fasciata
	Rhodophyta	*Corallina officinalis*
		Gelidium pulchellum
		Jania rubens
January (2018) Winter	Chlorophyta	*Ulva fasciata*
		Ulva compressa
		Chaetomorpha linum
		Codium arabicum
	Rhodophyta	*Amphiroa fragilissima*
		Corallina mediterranea
		Corallina officinalis
April (2018) Spring	Chlorophyta	*Ulva fasciata*
		Ulva compressa
	Rhodophyta	*Amphiroa fragilissima*
		Pterocladia capillacea
	Phaephyta	*Padina tetrastromatica*
		Petalonia fascia
July 2018 Summer	Chlorophyta	*Ulva fasciata*
		Cladophora glomerata
	Rhodophyta	*Pterocladia capillacea*
		Hypnea musciformis
		Gracilaria bursa-pastoris
	Phaephyta	*Padina tetrastromatica*

Table 1
Seasonal variations in the environmental conditions and water composition at the collection site in different seasons (2017–2018).

Parameter	Seasons			
	Autumn	Winter	Spring	Summer
Physical Parameter				
Temperature (°C)	24.00 ± 1.53[a]	20.20 ± 0.10[b]	20.50 ± 0.15[b]	25.00 ± 1.00[a]
Humidity%	25.00 ± 1.00[b]	30.00 ± 1.53[a]	30.00 ± 1.00[a]	20.00 ± 0.58[c]
PH	7.25 ± 0.03[a]	6.00 ± 0.58[a]	6.00 ± 0.58[a]	7.00 ± 1.15[a]
Color	Colorless	Colorless	Colorless	Colorless
Turbidity (NTU)	5.50 ± 0.05[ab]	4.00 ± 1.00[b]	6.00 ± 0.58[a]	4.00 ± 0.0.58[b]
Conductivity (μmhos cm^{-1})	28333.30 ± 19.05[d]	65000.00 ± 57.73[a]	54250.00 ± 28.87[c]	62400.00 ± 57.74[b]
T.D.S (mg L^{-1})	17,000 ± 100.00[d]	39000.00 ± 115.47[a]	32550.00 ± 5.77[c]	37440.00 ± 11.55[b]
Chemical parameter				
Dissolved anions (mg L^{-1})				
Chloride Cl^-	17100.00 ± 100.00[d]	23700.00 ± 40.41[a]	19460.00 ± 41.63[c]	21300.00 ± 35.12[b]
Sulfate SO_4^{-2}	496.00 ± 16.29[a]	400.00 ± 10.00[b]	100 ± 20.00[c]	20.00 ± 5.00[d]
Bicarbonate HCO_3^{-2}	6785.00 ± 32.53[a]	904.00 ± 7.2[d]	3324.70 ± 57.73[b]	1845.00 ± 50.08[c]
Nitrate NO_3^-	4.30 ± 0.06[a]	0.20 ± 0.01[c]	0.10 ± 0.02[c]	2.99 ± 0.06[b]
Nitrite NO_2^-	0.28 ± 0.02[a]	0.007 ± 0.00[c]	0.003 ± 0.00[c]	0.006 ± 0.00[c]
Dissolved cations (mg L^{-1})				
Ammonium NH_4^+	26.70 ± 0.05[a]	2.70 ± 0.12[c]	0.95 ± 0.02[d]	4.90 ± 0.17[b]
Calcium Ca^{++}	1152.60 ± 0.06[c]	4656.00 ± 3.46[a]	814.00 ± 1.00[d]	3600.00 ± 26.45[b]
Magnesium Mg^{++}	2567.40 ± 0.10[a]	942.84 ± 0.06[d]	2.89[c]±1485.00	1800.00 ± 10.00[b]
Salinity	30000.00 ± 100.00[d]	39000.00 ± 152.75[a]	32550.00 ± 100.00[c]	37440.00 ± 50.33[b]
Total Hardness as $CaCO_3$	8646.40 ± 3.96[c]	15520.00 ± 100.00[b]	8148.00 ± 50.01[d]	16407.40 ± 76.37[a]

Data are expressed as the mean ± standard deviation (SD) of three replicates. Different letters represent the statistically significant between groups by using one-way ANOVA and post hoc Duncan's test ($p < 0.05$).

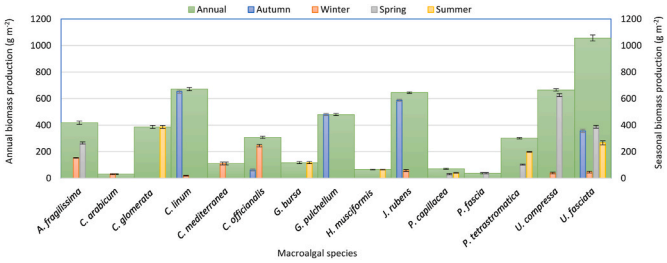

Fig. 1. Seasonal and annual biomass productivity of different collected macroalgal species.

Soando, and Haseom sites of the western and southern coasts of Korea.

On the other hand, *U. fasciata* was the dominant species by biomass along the different seasons. Although the seasonal growth of *U. fasciata* during all season was relatively lower than those recorded for *C. linum* and *U. compressa* in autumn and spring, it showed the highest annual biomass production of 1056.8 g m^{-2} due to semi-stable growth in all seasons. The annual biomass production of *U. fasciata* showed 57.5% and 58.9% higher than that of *C. linum* and *U. compressa*, respectively.

3.3. Biochemical composition

The seasonal variations in the collected seaweeds' biochemical composition on a dry weight basis (% dw) are summarized in Table 3. Carbohydrates, proteins, and lipids are the most important biochemical components in the algal biomass. Carbohydrate considers the most important component for metabolism, and it supplies the energy needed for all metabolic processes (Banerjee et al., 2009; Elshobary et al., 2020a,b). From the present study, there are changes in carbohydrate content during different seasons, whereas the highest carbohydrate content was observed in *Hypnea musciformis* (25.02% dw) during summer, while the lowest value was recorded in *Amphiroa fragilissima* (4.99% dw) during spring (Table 3). The results show that protein content in the collected species during summer was somewhat higher than that in other seasons (Table 3). In the present study, the protein content of macroalgae collected in different seasons ranged between 0.95 and 20.77% dw. The maximum protein content was recorded in *H. musciformis* during summer, while the minimum was found in *C. glomerata* during autumn (Table 3). In a previous study, the protein content of macroalgae varied between 3 and 47% dw, depending on the phylum and species (Fleurence, 1999).

Regarding lipid content as the main content important for the present study as a biodiesel feedstock, it showed relatively lower values compared to carbohydrates and proteins. Lipid content varied among species (Table 3), with the highest value of 14.66% and 9.94% dw being in *U. fasciata and U. compressa* during spring, followed by *Padina tetrastromatica* (8.59%) during spring. Accordingly, the spring season showed the highest lipid content comparing to other seasons. Similar results were recorded previously for macroalgae collected during spring

Table 3
Biochemical composition of seaweeds collected in four different seasons. Data expressed as % per dry weight (% dw).

Seaweeds	Carbohydrate content (% dw)				Protein content (% dw)				Lipid content (% dw)			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
A. fragilissima	ND	7.38 ± 0.35^{d}	4.99 ± 0.48^{f}	ND	ND	4.640.08^{c}	4.18 ± 0.04^{e}	ND	ND	0.91 ± 0.01^{d}	0.76 ± 0.02^{e}	ND
C. arabicum	ND	15.91 ± 0.51^{c}	ND	ND	ND	9.44 ± 0.33^{a}	ND	ND	ND	5.07 ± 0.01^{a}	ND	ND
C. glomerata	ND	ND	ND	9.71 ± 0.46^{d}	ND	ND	ND	7.12 ± 0.11^{e}	ND	ND	ND	1.71 ± 0.04^{b}
C. linum	8.63 ± 0.63^{c}	23.40 ± 0.58^{a}	ND	ND	0.95 ± 0.024^{d}	5.67 ± 0.16^{c}	ND	ND	1.29 ± 0.01^{c}	4.97 ± 0.09^{b}	ND	ND
C. mediterranea	ND	6.67 ± 0.52^{d}	ND	ND	ND	3.96 ± 0.06^{d}	ND	ND	ND	0.88 ± 0.03^{e}	ND	ND
C. officinalis	6.50 ± 0.55^{d}	7.40 ± 0.27^{d}	ND	ND	2.54 ± 0.40^{c}	3.95 ± 0.09^{d}	ND	ND	0.87 ± 0.00^{c}	1.34 ± 0.01^{d}	ND	ND
G. pulchellum	ND	ND	ND	16.37 ± 0.62^{c}	ND	ND	ND	15.45 ± 0.57^{c}	ND	ND	ND	0.84 ± 0.04^{d}
G. pulchellum	20.24 ± 0.85^{a}	ND	ND	ND	6.33 ± 0.30^{a}	ND	ND	ND	2.55 ± 0.00^{a}	ND	ND	ND
H. musciformis	ND	ND	ND	25.02 ± 1.36^{a}	ND	ND	ND	20.77 ± 0.64^{a}	ND	ND	ND	0.28 ± 0.07^{e}
J. rubens	9.67 ± 0.53^{c}	ND	ND	ND	2.03 ± 0.29^{c}	ND	ND	ND	0.91 ± 0.00^{c}	ND	ND	ND
P. capillacea	ND	ND	20.37 ± 0.81^{b}	18.62 ± 0.73^{b}	ND	ND	11.46 ± 0.64^{c}	18.69 ± 0.11^{b}	ND	ND	1.59 ± 0.02^{d}	1.47 ± 0.06^{c}
P. fascia	ND	ND	13.77 ± 0.22^{d}	ND	ND	ND	14.51 ± 0.11^{a}	ND	ND	ND	3.51 ± 0.03^{c}	ND
P. tetrastromatica	ND	ND	22.50 ± 0.75^{a}	18.83 ± 0.68^{b}	ND	ND	13.09 ± 0.64^{b}	19.08 ± 0.20^{b}	ND	ND	8.59 ± 0.05^{b}	2.42 ± 0.09^{a}
U. compressa	ND	15.13 ± 0.44^{c}	11.33 ± 0.51^{e}	ND	ND	7.15 ± 0.20^{b}	3.91 ± 0.23^{e}	ND	ND	2.55 ± 0.02^{c}	9.94 ± 0.01^{b}	ND
U. fasciata	16.32 ± 0.74^{b}	20.93 ± 0.39^{b}	18.49 ± 0.61^{c}	18.80 ± 0.59^{b}	5.01 ± 0.55^{b}	3.77 ± 0.21^{d}	4.84 ± 0.15^{d}	12.24 ± 0.13^{d}	2.15 ± 0.01^{b}	1.34 ± 0.02^{d}	14.66 ± 0.04^{a}	0.88 ± 0.02^{d}

Data are expressed as the mean ± standard deviation (SD) of three replicates. Different superscript letters in the same column represent the statistically significant by using one-way ANOVA and post hoc Duncan's test ($p < 0.05$). ND, the corresponding seaweed was not detected at that season.

(El Maghraby and Fakhry, 2015; Khairy and El-Shafay, 2013). However, *H. musciformis* recorded the lowest lipid content of 0.28% dw during summer.

3.4. Lipid productivity and FAMEs profile

Lipid annual areal productivity is an essential parameter to select a promising biodiesel feedstock. It can be noted from Fig. 2 that the highest annual lipid productivity of 67.4 and 63.3 g m^{-2} was recorded with *U. fasciata and U. compressa*. It is mainly attributed to the simultaneous high lipid content (14.66% and 9.9% dw) in Spring (Table 3), which resulted in the highest areal seasonal lipid productivity in spring (56.8 and 62.3 g m^{-2}), respectively. The temporal variations in lipid content can be attributed to the genetic diversity, the abundance of the genus, and seasonal changes in the environmental parameters over different seasons, which were also reported to affect fatty acid profile (Boulom et al., 2014; Osman et al., 2020; Susanto et al., 2019). In this context, the total lipid content of the six seaweeds collected from the Red sea of Egypt was varied from 0.6% dw in spring to 1.1% dw in winter, which was correlated with nitrite and pH value (El-Manawy et al., 2019). Seasonal and environmental factors can alter the total lipid content, including glycolipids, phospholipids, and betaine lipids in *Fucus vesiculosus* by varying the fatty acid profile (Susanto et al., 2019). Therefore, studying the fatty acid profile of the different collected species is of great importance to further evaluate biodiesel quality.

Table 4 shows the fatty acid profile of each seaweed in different seasons. The current study demonstrated that marine algae exposed to seasonal fluctuations exhibit various concentrations of total, saturated, and unsaturated fatty acids, each with a characteristic profile. These results agree with that of Khairy and El-Shafay (2013), who reported that the fatty acid composition of different seaweeds collected from the same collecting area was varied in different seasons. The environmental and seasonal factors significantly impacted the contents of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and total fatty acids in both blade and sporophyll of *Undaria pinnatifida*, in the Marlborough Sounds, New Zealand (Boulom et al., 2014).

In all species, 16-carbon and 18-carbon fatty acids showed the dominant fatty acids, considered the optimum carbon chain length for biodiesel (Knothe, 2008). In general, SFAs content showed the highest proportion among all species except *C. glomerata*. Khairy and El-Shafay (2013) and Elshobary et al. (2020) found that SFAs was a dominant component in most Mediterranean seaweeds. Among all seaweed species, green seaweeds *U. compressa and U. fasciata* during spring represented the highest SFAs (22.63 and 19.33 mg g^{-1} dw), which represented 71.8 and 60% of total fatty acids, respectively, while red seaweed *H. musciformis* of summer showed the lowest SFAs of 1.24 mg g^{-1}dw. This finding was in accordance with (Osman et al., 2020), who stated that *Ulva* species showed the highest SFAs among 22 species collected from the Alexandria coast. By contrast, Gosch et al. (2012) demonstrated that PUFAs are most common in the green macroalgae but are less in the brown and red macroalgae. Regarding unsaturated fatty acids, *P. tetrastromatica* of spring showed the greatest MUFAs of 10.66 mg g^{-1} dw, according to oleic acid's dominance (C18:1). However, *C. glomerata* recorded the highest polyunsaturated fatty acids (PUFAs) of 8.30 mg g^{-1} dw during summer due to the dominant of C18:2 and C18:3 fatty acids. Similar results were recorded by (Gubelit et al., 2015), who observed that PUFAs content of *C. glomerata* collected from the Baltic Sea of Finland was higher than SFA according to the dominant of C18:2, C18:3, and C20:5 fatty acids. Palmitic acid (C16:0) showed the highest value among all fatty acids in all studied species, and the highest content was observed in *U. compressa* and *U. fasciata* (19.62 and 16.85 mg g^{-1} dw), which represented 87% of SFAs. Therefore, saturated fatty acids (SFAs) showed a higher concentration (1.24–22.63 mg g^{-1} dw) over MUFAs or PUFAs in all species. In terms of quality, high content of unsaturated fatty acids is not preferred in because of the high oxidation stability and avoid cold flow problems (Krzemińska and Oleszek, 2016; Song et al., 2013). Thus, the high percentage of saturated fatty acids, to a certain extent, was reported to have a positive impact on the transesterification process and biodiesel quality (Hu et al., 2008). Moreover, the high content of C16–C18 improves the biodiesel quality and performance (Elshobary et al., 2020a,b; Huo et al., 2020). The highest C16–C18 content was recorded in *P. tetrastromatica* during spring (29.7 mg g^{-1}dw), followed by green seaweeds of *U. compressa* and *U. fasciata* during spring (28.51 and 22.10 mg g^{-1} dw, respectively), and the lowest content was observed in *H. musciformis* during summer. Within unsaturated fatty acids, oleic acid (C18:1) and palmitoleic acid (C16:1) supply the optimal compromise between oxidative stabilization and cold flow. (Hoekman et al., 2012; Song et al., 2013). Overall, the high contents of C16–C18 and SFAs and low contents of MUFAs and PUFAs, ensure excellent biodiesel performance (Elshobary et al., 2020a,b; Huo et al., 2020). Therefore, current results confirmed that the fatty acid profile of FAMEs produced from all species can be suitable for production of biodiesel, and, thus, biodiesel quality was estimated.

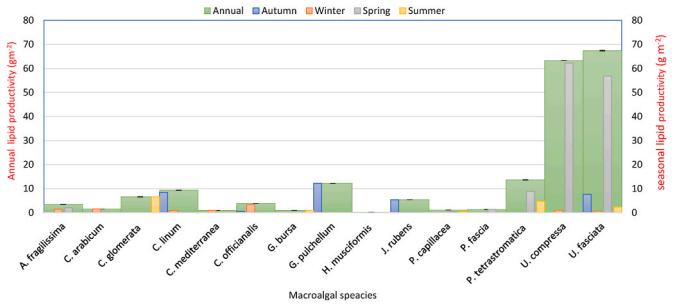

Fig. 2. Seasonal and annual areal lipid productivity of the collected seaweeds.

3.5. Biodiesel characteristics

The appropriate quality of the FAMEs produced is a critical parameter for the effective use of biodiesel produced, which is mainly affected by the profile of fatty acid (Ashour et al., 2019; Sebestyén et al., 2020). Table 5 shows the main biodiesel properties of the studied seaweeds in comparison with the international standards of the American Society for Testing and Materials (ASTM 6751−08) (D6751-08, 2008) and European Standards (EN 14214) (Fuels, 2008). Biodiesel characteristics, specially KV, CN, and IV were reported as the most common useful parameters for biodiesel evaluation (Yodsuwan et al., 2017). Generally, SG, CN, and IV of all species were within the ranges specified by the international standards (D6751-08, 2008; Fuels, 2008)). However, *H. musciformis* showed a higher KV value (5.11 mm^2 s^{-1}) than those recommended by the ASTM standard (up to 5.0 mm^2 s^{-1}), which is attributed to the absence of PUFA. Cetane number reflects the ignition quality, oxidative stability, and SFAs/USFAs ratio of the biodiesel (Elshobary et al., 2019; Hoekman et al., 2012; Song et al., 2013). The minimum recommended CN value is 47 (Fuels, 2008), while all studied species showed higher CN (Table 5). The relatively high cetane number observed in the this study makes the combustion efficiency biodiesel competitive to fossil diesel (Knothe, 2016). In addition, the low iodine value enhances oxidative stability during prolonged storage (Battah et al., 2015; Yodsuwan et al., 2017). Interestingly, the promising green seaweeds *U. compressa* and *U. fasciata* in the lipid content and fatty acid composition showed high relative CN during spring (59.73 and 55.12, respectively) and the IV within the range of international standards of less than 120 g I_2.100 g^{-1} OIL. All the studied species showed HHV numbers within the range of 38.8–42.05 Mj $kg^{-1,}$ which is greater than those estimated for seaweeds in previous studies (Abomohra et al., 2018; Osman et al., 2020). From the energy point of view, the net energy output was calculated for the most promising species of *U. fasciata* and *U. compressa* by multiplying the biodiesel yield (total fatty acid content)

Table 4
Fatty acid profile of the collected seaweeds calculated by mg g^{-1} dw at different seasons.

Fatty acids	*A. fragilissima*		*C. arabicum*	*C. glomerata*	*C. linum*		*C. mediterranea*	*C. officinalis*		*G. bursa*	*G. pulchellum*	*H. musciformis*	*J. rubens*	*P. capillacea*		*P. fascia*	*P. tetrastromatica*		*U. compressa*		*U. fasciata*			
	[a]Wi	Sp	Wi	Su	Au	Wi	Wi	Au	Wi	Su	Au	Su	Au	Sp	Su	Sp	Sp	Su	Wi	Sp	Au	Wi	Sp	Su
C10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.43	0.00	0.00	0.73	0.00
C12:1	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.76	0.00
C13:0	0.13	0.18	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.18	2.58	0.00	0.04	1.75	0.00
C14:0	0.00	0.00	0.37	0.00	0.50	0.00	0.28	0.20	0.20	0.00	0.60	0.00	0.24	0.61	0.39	1.39	0.00	0.52	0.00	0.00	0.35	0.09	0.00	0.12
C15:0	0.04	0.00	0.00	0.00	0.03	0.00	0.08	0.05	0.06	0.31	0.05	0.00	0.03	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
C16:0	3.05	2.30	8.99	0.00	3.49	11.96	3.05	2.61	2.29	2.45	6.31	1.12	3.03	4.62	3.79	6.40	16.37	4.54	6.41	19.62	5.46	4.00	16.86	3.39
C16:1	0.11	0.06	0.44	0.00	0.33	0.25	0.16	0.14	0.20	0.11	0.25	0.08	0.21	0.10	0.26	0.00	1.44	0.95	0.84	0.46	0.50	0.00	0.43	0.14
C16:2	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C17:0	0.08	0.00	0.10	0.00	0.00	0.00	0.00	0.04	0.00	0.03	0.04	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C17:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C18:0	0.11	0.09	0.22	0.00	0.12	0.00	0.11	0.22	0.16	0.13	0.25	0.00	0.21	0.27	0.17	0.00	0.00	0.23	0.00	0.00	0.43	0.11	0.00	0.21
C18:1	0.34	0.72	6.12	2.31	1.45	2.26	0.35	0.51	0.89	0.34	1.01	0.14	0.38	0.95	1.10	5.05	9.23	2.74	1.19	4.60	2.82	0.74	4.81	0.36
C18:2	0.06	0.17	0.88	4.89	0.33	1.16	0.08	0.13	0.12	0.07	0.11	0.00	0.07	0.09	0.18	1.38	0.00	0.55	0.61	1.67	0.59	0.21	0.00	0.06
C18:3	0.00	0.31	2.16	3.41	0.00	3.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	1.74	2.14	0.00	0.58	0.00	0.00
C18:4	0.00	0.00	0.00	0.00	0.07	1.35	0.00	0.03	0.12	0.00	0.00	0.00	0.00	0.00	0.21	0.00	2.04	0.97	0.76	0.00	0.14	0.66	0.00	0.14
C20:0	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.03	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00
C20:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C20:2	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00
C20:4	0.15	0.00	0.54	0.00	0.04	0.29	0.25	0.15	0.47	0.39	1.73	0.00	0.10	0.13	0.23	0.00	1.88	0.63	0.14	0.00	0.00	0.10	1.28	0.00
C20:5	0.43	0.00	0.24	0.00	0.00	0.00	0.00	0.24	1.80	0.35	1.06	0.00	0.09	0.84	0.76	1.76	0.00	0.13	0.00	0.00	0.00	0.00	5.05	0.00
C22:0	0.00	0.00	0.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.07	0.00	0.00
C24:0	0.00	0.00	0.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C16–C18	3.70	3.65	18.82	16.30	5.79	20.27	3.76	3.63	3.79	3.10	7.93	1.34	3.89	6.03	5.69	12.83	29.07	10.04	11.55	28.51	9.94	6.29	22.10	4.30
[b]**SFAs**	3.40	2.57	10.65	0.00	4.18	12.11	3.52	3.14	2.72	2.92	7.33	1.24	3.60	5.50	4.34	7.79	16.37	5.50	6.69	22.63	6.24	4.31	19.33	3.72
MUFAs	0.45	0.77	6.69	2.31	1.78	2.51	0.51	0.67	1.18	0.45	1.26	0.22	0.69	1.05	1.35	5.05	10.66	3.68	2.03	5.07	3.32	0.78	6.00	0.50
PUFAs	0.67	0.48	3.81	8.30	0.44	6.23	0.33	0.54	2.60	0.82	2.90	0.00	0.26	1.05	1.38	3.15	3.92	2.35	3.26	3.82	0.73	1.54	6.89	0.20
TFAs	4.53	3.83	21.16	10.61	6.39	20.84	4.36	4.35	6.49	4.18	11.49	1.47	4.54	7.61	7.07	15.99	30.96	11.54	11.98	31.52	10.29	6.63	32.22	4.43

[a] Season with no detectable seaweeds is not shown in the table. Au, autumn; Wi, winter; Sp, spring; Su. Summer.
[b] SFAs, Saturated fatty acid; MUFAs monounsaturated fatty acids; PUFAs polyunsaturated fatty acids; TFAs total fatty acids.

Table 5
Comparison of the estimated biodiesel characteristics of the collected seaweeds with the international biodiesel standards.

	A. fragilissima		*C. arabicum*	*C. glomerata*	*C. linum*		*C. mediterranea*	*C. officinalis*		*G. bursa pulchellum*	*H. musciformis*	*J. rubens*	*P. capillacea*		*P. fascia*	*P. tetrastromatica*		*U. compressa*	*U. fasciata*				ASTM D6751-08	EN 14,214
	[a]Wi	Sp	Wi	Su	Au	Wi	Wi	Au	Wi	Su	Au	Su	Au	Sp	Su	Sp	Su	Wi	Sp	Au.	Wi.	Sp.	Su.		
ADU[b]	0.75	0.54	0.86	0.56	0.45	1.03	0.38	0.65	2.00	0.94	1.19	0.15	0.37	0.78	1.03	1.04	0.85	1.04	1.01	0.47	0.49	0.90	1.16	0.27	–	–
KV	4.73	4.87	4.66	4.85	4.92	4.55	4.97	4.80	3.95	4.61	4.45	5.11	4.97	4.72	4.56	4.55	4.67	4.55	4.57	4.91	4.90	4.64	4.47	5.04	1.9–6.0	3.5–5.0
SG	0.88	0.87	0.88	0.88	0.87	0.88	0.87	0.88	0.88	0.88	0.88	0.87	0.87	0.88	0.88	0.88	0.88	0.88	0.88	0.87	0.87	0.88	0.88	0.87	0.85–0.9	
Cp	9.99	12.82	8.46	12.45	14.01	6.20	14.93	11.33	−6.68	7.47	4.07	17.96	15.04	9.60	6.27	6.10	8.62	6.05	6.52	13.70	13.44	8.00	4.46	16.37	–	–
CN	57.88	59.29	57.12	59.11	59.89	55.99	60.35	58.55	49.56	56.62	54.93	61.86	60.40	57.68	56.02	55.94	57.20	55.91	56.15	59.73	59.60	56.89	55.12	61.07	Min. 47	51–120
IV	68.40	52.67	76.91	54.73	46.01	89.54	40.93	60.96	161.22	82.46	101.37	24.02	40.30	70.61	89.13	90.07	76.03	90.35	87.72	47.75	49.23	79.48	99.22	32.87	–	≤120
HHV	39.85	39.48	40.05	39.53	39.32	40.35	39.20	39.68	42.05	40.18	40.63	38.80	39.19	39.90	40.34	40.36	40.03	40.37	40.31	39.36	39.40	40.11	40.58	39.01	–	–
NEO	0.18	0.15	0.85	0.64	0.25	0.84	0.17	0.17	0.27	0.17	0.47	0.06	0.18	0.30	0.29	0.65	1.24	0.47	0.48	1.24	0.41	0.27	1.31	0.17		

[a] Season with no detectable seaweeds is not shown in the table. Au, autumn; Wi, winter; Sp, spring; Su. Summer.
[b] Characteristics included the average degree of unsaturation (ADU, %), kinematic viscosity (KV, $mm^2 s^{-1}$), specific gravity (SG, kg^{-1}), cloud point (CP, °C), cetane number (CN), iodine value (IV, g I_2 100 g^{-1} oil), higher heating value (HHV, MJ kg^{-1}), and net energy output (NEO, MJ ton^{-1}).

by the HHV of each product. The net energy outputs were ranged from 0.056 to 1.31 GJ ton^{-1}. The highest net energy output was recorded in *U. fasciata, U. compressa,* and *P. tetrastromatica* (1.30, 1.24, and 1.24 GJ ton^{-1}, respectively) during the spring season, which is comparable with those estimated for *U. intestinalis* (Osman et al., 2020)or *Dilophus fasciola* (Elshobary et al., 2020a,b) collected from the Mediterranean Sea. It is important to mention that these are simplified calculations where energy input required in collecting, transport, dewatering, and drying was not deemed. As wild seaweeds are naturally growing organisms that do not require costly nutrient or chemical fertilizers for cultivation. In addition, seaweeds were manually collected with no need for complex technical procedures for drying, reducing the gross production cost. Overall, these seaweeds collected from Abu Qir Bay, Alexandria, Egypt, presented qualified biodiesel characteristics, comparable energy output, and cost-effective production that could compete with fossil fuel.

4. Conclusion

Seaweeds were collected from Abu Qir Bay in Egypt's northern Mediterranean coastline area over different season for this study. *Ulva fascia* showed the highest annual biomass production, followed by *Ulva compressa*. Aggregating all together, *U. fasciata* and *U. compressa* with the highest lipid productivity, FAMEs composition, and biodiesel characteristics that comply with the international standards as well as high energy output that strongly support this species as a promising biodiesel feedstock. The availability of these seaweeds, mostly in spring, gives them the advantage of lower harvesting and labor cost that could only be applied in a single season. However, further economic analysis is required to study the process feasibility and life cycle assessment.

Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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