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ORIGINAL RESEARCH ARTICLE

Lipid content and fatty acid composition of Mediterranean macro-algae as dynamic factors for biodiesel production☆

Dahlia M. El Maghraby, Eman M. Fakhry*

Department of Botany and Microbiology, Faculty of Science, Alexandria University, Alexandria, Egypt

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Summary Using the total lipid contents and fatty acid profiles, the marine macro-algae *Jania rubens* (Rhodophyceae), *Ulva linza* (Chlorophyceae) and *Padina pavonica* (Phaeophyceae) were evaluated for biodiesel production during the spring, summer and autumn. Seawater parameters such as pH, salinity and temperature were measured. The total lipid content varied from 1.56% (*J. rubens*) to 4.14% (*U. linza*) of dry weight, with the highest values occurring in spring. The fatty acid methyl ester profiles were analysed using gas chromatography. The highest percentage of total fatty acids was recorded in *P. pavonica*, with 6.2% in autumn, whereas the lowest was in *J. rubens*, with 68.6% in summer. The relative amount of saturated to unsaturated fatty acids was significantly higher in *P. pavonica* than in the other macro-algae. Seasonal variations in pH, salinity and temperature had no significant effect on the total lipid and fatty acid contents. Principal component analysis grouped brown and green algae together, whereas red alga grouped out. Furthermore, methyl ester profiles indicate that brown and green seaweeds are preferred, followed by red seaweeds, which appears to have little potential for oil-based products. Therefore, these seaweeds are not targets for biodiesel production.

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* Corresponding author at: Department of Botany and Microbiology, Faculty of Science, Alexandria University, 21511 Alexandria, Egypt. Tel.: +20 1220338104; fax: +20 1006231928.
E-mail address: emfakhr@hotmail.com (E.M. Fakhry).
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1. Introduction

Energy is the most essential requirement for human survival. The complete dependence of mankind on fossil fuels may cause a major shortage in the future. Biofuels made from bio-products reduce the need for petroleum oil and offer considerable benefits for sustainability and reduce pollutant and greenhouse gas emissions (Hansen et al., 2009). Of the biofuels, biodiesel is highly promising. The main

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advantages of using biodiesel are that it is renewable, non-toxic, and biodegradable and can be used without modifying existing engines because it possesses similar properties to diesel fuel and produces less harmful gas emissions, such as sulphur oxide (Agarwal, 2007; Hansen et al., 2009). Biodiesel reduces net carbon dioxide emissions by 78% on a lifecycle basis compared to conventional diesel fuel (Gunvachai et al., 2007). Biodiesel consists of fatty acid methyl esters prepared from triglycerides by transesterification with methanol (Gerpen, 2005). During transesterification, the glycerides in fats or oils react with an alcohol in the presence of a catalyst (Banerjee and Chakraborty, 2009; Enweremadu and Mbarawa, 2009; Zabeti et al., 2009) and are converted into monoesters, yielding free glycerol as a by-product.

Biodiesel can be produced from different feedstocks. Each originating oil or fat is characterised by a different fatty acid composition, and the final ester properties differ significantly based on the feedstock, alcohol used in the esterification and the exact chemical process followed (Knothe, 2005). Recently, much research has focused on the production of biodiesel from non-edible sources, such as *Jatropha* and algae (Komninos and Rakopoulos, 2012; Pinzi et al., 2009). There has been increased interest in the marine production of biofuels derived from macro-algae (seaweed) and microalgae (single cell plants) (Singh and Cu, 2010; Williams and Laurens, 2010). Biodiesels derived from micro- and macro-algae have become known as one of the most encouraged unusual sources of lipids for use in biodiesel production because they are renewable in nature, can be produced on a large scale and are environmentally friendly (Carvalho et al., 2011). Macro-algae are multicellular, macroscopic algae, which are abundant in coastal environments, primarily in near-shore coastal waters with suitable substrates for attachment (Murphy et al., 2013). Seaweed is one of the best growing plants worldwide. It does not require irrigation or fertilisers, and it does not require arable land. A previous study reported that seaweed species have total lipid contents of less than 5% dry weight. By contrast, there are many species with total lipid contents greater than 10% dry weight, and these are interesting candidates for oil-based products (Gosch et al., 2012). Because fossil fuel prices are likely to increase and because macro-algal production costs will likely decrease as production is expanded, it is prudent to develop methods to obtain significant quantities of biofuel from marine biomass to meet European energy needs and climate change targets (Hughes et al., 2012).

The objective of this study was to assess the potential of *Jania rubens* (Rhodophyceae), *Ulva linza* (Chlorophyceae) and *Padina pavonica* (Phaeophyceae) that inhabit the Abu Qir Bay coast, Alexandria, Egypt, for biodiesel production. The quantification of total lipid content and identification of fatty acid profiles for these species was performed. The total lipid content in relation to the fatty acid content for the macro-algae during different seasons was estimated. Additionally, the variation in the fatty acid profiles of these species between and within seasons was determined to identify the most favourable conditions to produce seaweeds with high lipid contents and optimal fatty acid profiles.

2. Material and methods

2.1. Area of study and sampling

Seaweed species belonging to different classes, including *J. rubens* (Rhodophyceae), *U. linza* (Chlorophyceae) and *P. pavonica* (Phaeophyceae), were collected seasonally through the spring, summer and autumn from Abu Qir Bay. Winter showed a quantitative reduction in algal flora. The samples were identified based on the morphological features using the herbarium and the identification scheme of the late Prof. A. H. Nasr (Botany Department, Faculty of Science, Alexandria University). Abu Qir Bay is a semi-circular bay along the Egyptian Mediterranean seashore, approximately 30 km east of Alexandria, with an average water depth of 11 m and an area of approximately 360 km^2. This bay is characterised by the presence of abundant rocks with several petite and fine holes that are excellent domains for the attachment of algae.

Algal thalli were placed separately in plastic bags, stored in an icebox and transported to the laboratory. They were washed thoroughly with tap water to remove any impurities. The water was drained off, and the algae were spread on filter paper to remove the excess water. The weighed samples were dried until they reached a constant weight. These shade-dried samples were ground into a fine powder. The original weight decreased approximately 10 times. Therefore, 1 kg wet seaweed will weigh 100 g (10 to 1 wet to dry ratio).

2.2. Water measurements

During three successive seasons, namely spring, summer and autumn, seawater samples were collected using clean glass bottles for the field measurements. The pH of the water samples was measured using a digital pocket pH metre (FG-211). The salinity was measured with an inductive salinometer (model MI-150). The water temperature was measured with a mercury thermometer graduated to 0.1°C.

2.3. Lipid extraction and fatty acid analysis

The seaweed samples were analysed in triplicate for their proximate lipid content using the Bligh and Dyer (1959) method. Samples were homogenised with a 1:2 mixture of chloroform and methanol and incubated in the dark overnight. The residues were extracted 2—3 times with a small amount of chloroform and methanol. The chloroform layer was removed with a separating funnel and then vaporised in an evaporator. The lipid content was calculated by weighing the residues and was expressed as a percentage of dry weight. The fatty acids were converted to methyl esters using the method of Christie (1998). The samples were esterified in 1% sulphuric acid in absolute methanol and extracted with hexane to separate the layers. The hexane layer was washed with water containing potassium bicarbonate and dried over anhydrous sodium sulphate. The solvent was evaporated using a rotary evaporator. The fatty acid methyl esters (FAMEs) were analysed on a Shimadzu gas-liquid chromatographer equipped with a flame ionisation detector with a packing column with Hp-5 material. The carrier gas was nitrogen, and the short speed was 5 mm/min.

For the identification and quantification of FAMEs, their retention times were compared with standards. The values are expressed as a percentage of the total fatty acids mixture.

2.4. Statistical analysis

The variation in fatty acid composition between the species during different seasons was evaluated using principal component analysis (PCA) (SPSS IBM version 20) with the mean of three individual samples as the variables. Three principal component analyses (PCAs) were performed separately on the total, saturated and unsaturated fatty acids. The outcome was plotted in two dimensions (PCA1, PCA2). The score loading was analysed and identified in the bi-plot of PCA1 versus PCA2.

3. Results

3.1. Water characteristics

The seawater parameters, such as pH, salinity and temperature, showed limited variations during the different seasons (Table 1). The pH value varied between a maximum of 8.11 during spring and a minimum of 7.60 during summer, whereas the pH value during autumn was in between (7.78). The average values of water salinity decreased in the order of autumn (32.15 g/L), spring (36.21 g/L) and summer (38.32 g/L). The seasonal temperature variations followed the climate conditions. There was significant variation between the seasons, with the highest values during the summer (29.30°C). Furthermore, the lowest values fluctuated between 21.50°C and 20.90°C in the autumn and spring, respectively.

3.2. Lipid content and fatty acid distribution

The seasonal variations in the total lipid content based on the dry weight of *J. rubens* are shown in Table 2. The highest lipid content was 2.51% in the spring, followed by 2.42% in the summer, and the lowest value was 1.56% in autumn. The fatty acid methyl esters as a percentage of the total fatty acids mixture are shown in Table 2. The total percentage of identified saturated fatty acids was 40.53, 31.45 and 38.92% and for the unsaturated fatty acids was 37.29, 37.17 and 51.54% in the spring, summer and autumn, respectively, with approximate ratios between the saturated and unsaturated fatty acids of 1.09, 0.85 and 0.76. For the individual fatty acids, the major saturated fatty acids were myristic acid (C13:0) and palmitic acid (C16:0) in both the spring and summer, whereas pentadecyclic acid (C15:0) and palmitic acid (C16:0) were the major saturated fatty acids in autumn. By contrast, docosahexaenoic acid (C22:6) and pentadecenoic acid (C15:1) were the major unsaturated fatty acids during the different seasons.

Table 3 shows the variation in total lipid content of *U. linza* in the spring, summer and autumn. The highest percentage was 4.14% of dry matter in the spring. Comparable percentages of 3.76 and 3.20% were observed in the summer and autumn, respectively. Table 3 also shows an overview of the fatty acid profiles of the alga. In this study, we identified several individual fatty acids during various seasons with different concentrations. The saturated fatty acids were primarily C16:0, with 56.13, 38.10 and 48.44% in the spring, summer and autumn, respectively. By contrast, the unsaturated fatty acids were mainly C22:6, with 9.16, 10.05 and 4.82%, and C15:1, with 4.92, 3.60 and 0.099% in the spring, summer and autumn, respectively. The sum of the saturated fatty acids of these seasons was 71.42, 51.20 and

Table 1 Seasonal variations of pH, salinity and temperature of the water of Abu-Qir Bay.

	Spring	Summer	Autumn
pH	8.11	7.60	7.78
Salinity (g/L)	36.21	38.32	32.15
Temperature (°C)	20.90	29.30	21.50

Table 2 Total lipid contents and fatty acid profiles of *Jania rubens* during different seasons.

	Season		
	Spring	Summer	Autumn
Total lipid[a]	2.51	2.42	1.56
Fatty acids[b]			
C8:0	0.27	0.26	0.60
C10:0	0.01	0.01	0.01
C12:0	2.26	2.16	4.35
C13:0	11.18	12.38	0.56
C14:0	6.19	1.58	3.12
C15:0	6.99	0.76	12.95
C16:0	8.46	10.86	12.87
C17:0	1.35	0.73	1.12
C18:0	2.86	1.31	2.29
C20:0	0.96	1.40	1.05
Sum of saturated	40.53	31.45	38.92
C15:1	9.07	9.56	16.39
C16:1	3.71	4.82	7.02
C17:1	0.39	0.17	0.29
C18:1	0.01	0.61	0.66
C18:2	5.08	0.23	0.43
C18:3	2.15	0.30	0.07
C20:1	—	0.61	0.01
C20:3	0.39	1.34	1.36
C20:4	1.10	0.28	—
C20:5	0.19	0.26	0.41
C22:1	0.25	0.20	0.01
C22:6	14.95	18.79	24.89
Sum of unsaturated	37.29	37.17	51.54
Total	77.82	68.62	90.46
SFA/UFA[c]	1.09	0.85	0.76
C16:1/C18:1/C14:0	0.5:0.002:1	3:0.4:1	2:0.2:1

All data were recorded as the mean of three replicates.
[a] Total lipids as a percentage of dry weight.
[b] Fatty acid methyl esters as a percentage of the total fatty acids mixture.
[c] SFA/UFA: ratio of saturated fatty acids to unsaturated fatty acids.

Table 3 Total lipid contents and fatty acid profiles of *Ulva linza* during different seasons.

	Season		
	Spring	Summer	Autumn
Total lipid[a]	4.14	3.76	3.20
Fatty acids[b]			
C8:0	0.05	0.25	0.18
C10:0	0.21	0.05	0.07
C12:0	1.68	0.75	0.68
C13:0	7.68	4.60	3.26
C14:0	1.23	1.25	1.78
C15:0	0.90	0.70	2.07
C16:0	56.13	38.10	48.44
C17:0	0.22	0.25	1.19
C18:0	1.27	3.80	4.99
C20:0	2.05	1.45	0.97
Sum of saturated	71.42	51.20	63.63
C15:1	4.92	3.60	0.99
C16:1	2.03	1.25	1.34
C17:1	0.14	0.25	1.41
C18:1	0.31	0.80	10.43
C18:2	0.17	1.15	0.99
C18:3	0.12	0.45	1.43
C20:1	0.17	0.10	0.33
C20:3	-	-	0.04
C20:4	0.05	0.80	-
C20:5	0.03	0.05	0.02
C22:1	1.21	1.10	3.10
C22:6	9.16	10.05	4.82
Sum of unsaturated	18.31	20.05	24.90
Total	89.73	71.25	88.53
SFA/UFA[c]	3.90	2.55	2.56
C16:1/C18:1/C14:0	2:0.3:1	1:0.6:1	1:6:1

All data were recorded as the mean of three replicates.
[a] Total lipids as a percentage of dry weight.
[b] Fatty acid methyl esters as a percentage of the total fatty acids mixture.
[c] SFA/UFA: ratio of saturated fatty acids to unsaturated fatty acids.

Table 4 Total lipid contents and fatty acid profiles of *Padina pavonica* during different seasons.

	Season		
	Spring	Summer	Autumn
Total lipid[a]	3.01	2.18	1.82
Fatty acids[b]			
C8:0	0.14	0.39	0.22
C10:0	0.32	0.07	0.10
C12:0	1.18	1.33	1.64
C13:0	4.28	5.08	6.84
C14:0	4.84	7.66	4.98
C15:0	0.95	2.92	3.98
C16:0	42.61	45.59	48.64
C17:0	0.70	0.71	0.58
C18:0	1.62	2.67	6.24
C20:0	1.74	0.94	1.04
Sum of saturated	58.38	67.36	74.26
C15:1	3.28	0.85	1.02
C16:1	1.57	1.54	2.34
C17:1	0.67	0.35	0.34
C18:1	0.92	4.28	2.48
C18:2	0.50	5.70	4.74
C18:3	0.39	0.14	0.22
C20:1	0.03	0.25	0.30
C20:3	0.19	0.81	0.54
C20:4	0.06	0.05	0.18
C20:5	0.70	1.22	0.48
C22:1	0.11	0.18	0.18
C22:6	5.99	6.12	8.84
Sum of unsaturated	14.41	21.49	22.02
Total	72.79	88.85	96.28
SFA/UFA[c]	4.05	3.23	3.37
C16:1/C18:1/C14:0	0.3:0.2:1	0.2:0.6:1	0.5:0.5:1

All data were recorded as the mean of three replicates.
[a] Total lipids as a percentage of dry weight.
[b] Fatty acid methyl esters as a percentage of the total fatty acids mixture.
[c] SFA/UFA: ratio of saturated fatty acids to unsaturated fatty acids.

63.63%, respectively, whereas the sum of the unsaturated fatty acids was 18.31, 20.05 and 24.90%, respectively.

The total lipid content of *P. pavonica* during different seasons is tabulated in Table 4. The lipid content in terms of dry weight was 3.01, 2.18 and 1.82% in the spring, summer and autumn, respectively. The fatty acid composition varied among the different seasons (Table 4). Autumn had the highest saturated fatty acid content as a percentage of the dry weight (74.26%), followed by summer (67.36%) and spring (58.38%). Moreover, similar results were obtained for the unsaturated fatty acid contents with a percentage of 22.02 in the autumn, 21.49 in the summer and 14.41 in the spring. The percentages of the saturated fatty acid C16:0 were 48.64, 45.59 and 42.61%, and the percentages of the unsaturated fatty acid C22:6 were 8.84, 6.12 and 5.99% from autumn to summer to spring, respectively.

3.3. Principal component analysis

Principal component analysis of the total fatty acids data, sum of the saturated fatty acids and sum of the unsaturated fatty acids demonstrated a statistical distinction between the three seaweeds. These algae showed high factor loading on PCA1 and PCA2. A bi-plot of the total fatty acids data matrix (Fig. 1a) explained 98.5% of the variances (64.5% and 34%). When PCA was applied to the saturated fatty acids (Fig. 1b), the model explained 99% of the total variances (62.4% and 36.5%). For the unsaturated fatty acids (Fig. 1c), PCA revealed 86% of the total variances (48% and 38%).

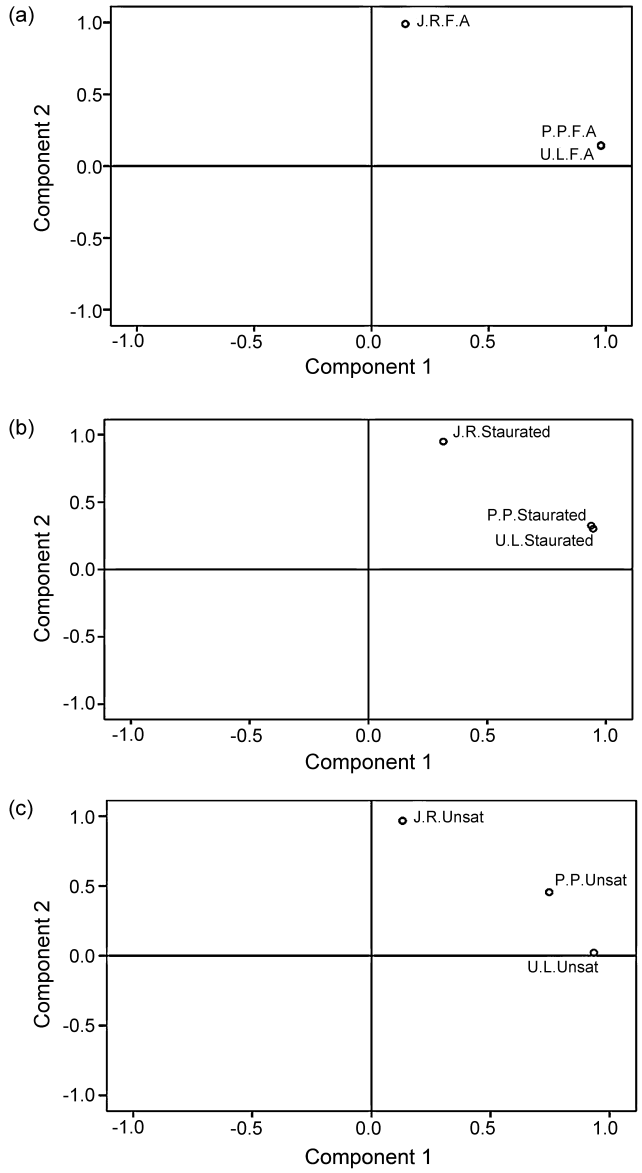

Figure 1 Bi-plot of the principal component analysis based on the (a) total fatty acids, (b) sum of saturated fatty acids and (c) sum of unsaturated fatty acids of the selected macro-algae (JRFA, PPFA, and ULFA: total fatty acids content of *Jania rubens*, *Padina pavonica* and *Ulva linza*, respectively; JRSaturated, PPSaturated, ULSaturated: sum of the saturated fatty acids in *J. rubens*, *P. pavonica* and *U. linza*, respectively; JRUnsat, PPUnsat, ULUnsat: sum of the unsaturated fatty acids in *J. rubens*, *P. pavonica* and *U. linza*, respectively).

4. Discussion

In the present study, the first step was to evaluate macro-algae as a feedstock for oil-based products to establish both qualitatively and quantitatively their total lipid content and fatty acid profiles. Subsequently, the extent to which environmental factors affect the total lipid content and fatty acid profiles of the algae under natural conditions was determined. These include pH, salinity (Juneja et al., 2013) and temperature (Graeve et al., 2002; Nelson et al., 2002).

In this study, the seasonal pH variations may be influenced by sewage discharge and the decomposition of organic matter because Abu-Qir Bay is subject to domestic sewage outfalls and industrial and agricultural effluents (Saad and Younes, 2006). By contrast, the seasonal variation in average salinity may be a result of high solar energy in the shallow water bay during summer compared to other seasons. This may be attributed to water evaporation because of elevated temperature. However, evaporation is a controlling factor for salinity. Environmental temperature affects algae and their habitat and may affect their lipid content and fatty acid patterns (Holton et al., 1964). In the present study, the small variation in temperature during the seasons had a slight effect on the algae. Furthermore, the maximum seasonal average temperature values occurred in summer, whereas the minimum occurred in spring and autumn. Because of the shallowness of the coastal water of Abu Qir Bay, thermal stratification was not frequently observed, except for some localities subjected to thermal pollution from industrial warm water discharge.

It is evident from this study that these seaweeds have low lipid content during all seasons. This is consistent with Jensen (1993), who reported that the lipid content is very low in seaweeds, ranging from 1 to 5% of the dry matter, and varies significantly between different algae. In this study, the green alga *U. linza* had the highest total lipid content, followed by the brown alga *P. pavonica* and the red alga *J. rubens*. These variations are likely because of the genetic diversity and temporal variations in the environmental parameters across different seasons. Additionally, it may be because of the abundance of the genus, which individually increased and showed maximum growth during several seasons and decreased during others. Accordingly, a low lipid content of these macro-algae decreases their utility for biodiesel production and emphasises that macro-algae are promising resources for other products. Murphy et al. (2013) suggested that the natural sugars and other carbohydrates contained in macro-algae make them suitable for biogas and ethanol production rather than biodiesel. By contrast, Gosch et al. (2012) evaluated macro-algae of the genus *Dictyota*, *Spatoglossum*, *Derbesia* and *Caulerpa* for lipids and reported a range from 10% to 12% of dry weight that is comparable with the micro-algae *Tetraselmis*, *Rhodomonas*, *Scenedesmus* and a few strains of *Skeletonema* and *Isochrysis* (Huerlimann et al., 2010; Mata et al., 2010). The authors suggest combining the macro-algae and using large amounts of raw materials to obtain a homogenous high lipid content, and accordingly these seaweeds could be exploited as a source of biodiesel.

The present study showed that marine algae subjected to seasonal variations exhibit different concentrations of total, saturated and unsaturated fatty acids, with a characteristic profile for each. This is expected for distant systematic relationships between these algae. Both *U. linza* and *P. pavonica* had the highest fatty acid percentages throughout the entire year compared to *J. rubens*. Palmitic acid (C16:0) was at relatively high concentrations. For *U. linza* and *P. pavonica*, palmitic acid comprised approximately 70%. For *J. rubens*, it comprised approximately 30% of the total saturated fatty acids for the studied seasons. This is a distinctive characteristic because palmitic acid (C16:0) is the primary saturated fatty acid in several seaweeds (Bemelmans et al., 2002; Denis et al., 2010; El-Shoubaky et al., 2008;

Khotimchenko, 1991; Matanjun et al., 2009). Simultaneously, docosahexaenoic acid (C22:6) presented with higher concentrations of unsaturated fatty acids in approximately 50% of these algae during the different seasons. However, for *U. linza* and *P. pavonica*, it was approximately 25% in autumn and summer, respectively. Gosch et al. (2012) reported that this essential polyunsaturated fatty acid is most common in the green seaweeds but is less in the brown and red seaweeds. By contrast, Khairy and El-Shafay (2013) found that it was a primary component in several macro-algae. Belarbi et al. (2000) and Chisti (2007) reported that algal oils differ from vegetable oils because they are relatively rich in polyunsaturated fatty acids with four or more double bonds, such as docosahexaenoic acid, which commonly occurs in algal oils.

For the ratios of saturated to unsaturated fatty acids in this study, *P. pavonica* exhibited the highest ratios (3.23, 3.37 and 4.05), followed by *U. linza* (2.55, 2.56 and 3.90), whereas *J. rubens* displayed relatively low ratios (0.85, 0.76 and 1.09) during the summer, autumn and spring, respectively. The principal component analysis shown in Fig. 1a—c separates these seaweeds based on their total, saturated and unsaturated fatty acids into two groups, with the brown and green seaweeds grouped together and the red seaweed grouped out.

However, quantification of the fatty acid components and varying degrees of saturation were significant factors in determining the suitability of these oils as biodiesel feedstock. Ramos et al. (2009) reported that monounsaturated, polyunsaturated and saturated methyl esters predict the critical parameters of the European standard for any biodiesel composition. For biodiesel production, algae with a high proportion of saturated fatty acids are preferred because this leads to higher oxidative stability and higher ignition quality (cetane number) and produces an overall higher quality product (Hu et al., 2008; Knothe, 2008). Furthermore, the fatty acid methyl ester profile is a key factor that determines the suitability of any feedstock for use in biodiesel fuel production (Knothe, 2009). For macro-algae biodiesel to be competitive with other biodiesel feedstocks, the ideal mixture of the fatty acids C16:1, C18:1 and C14:0 has been suggested to be in the ratio 5:4:1 (Schenk et al., 2008). In this study, Tables 2—4 show that none of these samples during any seasons achieved this significant ratio for target biodiesel production. Therefore, these seaweeds should be utilised for other purposes (Veena et al., 2007; Zemke-White and Ohno, 1999).

5. Conclusion

This study identified the total lipid and fatty acid contents of *J. rubens* (Rhodophyceae), *U. linza* (Chlorophyceae) and *P. pavonica* (Phaeophyceae) collected seasonally throughout spring, summer and autumn from Abu Qir Bay for biodiesel production. Although these algae displayed distinct variations in the total lipid content and fatty acid composition for all seasons, the overall amounts of total lipids were generally low, with a maximum content of 4.14% dry weight, which must be significantly increased for use in biodiesel production. Moreover, because the structural features of the various fatty esters determine the properties of biodiesel, the qualitative fatty acid yields of selected algae make them appropriate for products other than biodiesel.

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