# Lipid chemistry of green macroalgae *Ulva* sp. a potential resource for biotechnological applications in the Southern Mediterranean Sea Coast, Alexandria shore, Egypt

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#### **ABSTRACT**

The nutritional value and chemical changes in the lipid content of the green macroalgae, *Ulva sp.*, were assayed in the present study. *Ulva* samples were collected from two stations, Ras Al-Tin (station A) and El-Muntazah (Station B) along Alexandria shore on the Mediterranean Sea, in twelve sampling periods (from January to December 2012). Each three samples of each station were plotted to represent one season. The chemical characterization of the lipid fractions was performed by gas liquid chromatography. The result showed that *Ulva* lipid content is relatively high (9.4±1.5 and 12.2±2.7%DW, at station A and B respectively) that can be explained by the higher pollution level at the station A. The fatty acids (FAs) of Ulva sp. lipidic extract mainly composed of palmitic, oleic and linoleic FAs. Saturated fatty acids (SFAs) represented about 50% of the total FAs (TFAs). Monounsaturated fatty acids (MUFAs) accounted for a high percent, 17.6-33.4% of TFAs. Polyunsaturated fatty acids (PUFAs) existed in a high percent in *Ulva* extraction reaching a maximum in winter and spring at both stations, about 38.4 and 30.5% of TFAs at station A and B, respectively. The high percent of PUFA can be attributed to the correlation between the low temperature and the degree of unsaturation. Also, the long chain fatty acids (C16 and C18) constitute more than 82% of the TFAs, which are the main components for biofuel. The results indicate that *Ulva* has a good potential for its use in human and animal food and health maintenance as a rich source of MUFAs and PUFA as well as in biodiesel production. Also, the wide ranges of the fatty acids indicate the possibility of manipulating the fatty acids profile through the cultivation conditions.

Keywords: green macroalgae, Ulva, lipid chemistry, fatty acids profile

#### INTRODUCTION

Marine macroalgae are multicellular organisms with considerable potential for use as a bioactive compounds source of immense pharmaceutical value. They also show interesting nutritional characteristics because of their richness of nutritionally beneficial components such as proteins, polyunsaturated fatty acids (PUFAs), carbohydrates and antioxidants (Mohamed *et al.*, 2011; Kumari *et al.*, 2013a). However, the nutrient compositions of macroalgae vary depending on species, habitats, maturity and environmental conditions (Ito and Hori, 1989).

In general, macroalgae lipid content accounts for less than 5% of dry weight (DW) (Ortiz *et al.*, 2006; Van Ginneken *et al.*, 2011). Nevertheless, recent studies showed higher lipid content (up to 20% DW) in some green and brown macroalgae (Satpati and Pal, 2011; Rameshkumar *et al.*, 2012).

A distinctive property of the macroalgal lipid extract is that it contains higher levels of essential polyunsaturated fatty acids (PUFAs) including *n*-3 PUFAs compared with traditional vegetables (Ortiz *et al.*, 2006). The *n*-3 PUFAs are of

particular importance since they cannot be synthesized by humans or fish and are thus obtained only through dietary sources (Sanchez-Machado, *et al.*, 2004), as they may reduce the risk of heart disease, thrombosis and atherosclerosis (Van Ginneken *et al.*, 2011; Kumari *et al.*, 2013a). Moreover, it has also been reported that the fatty acids of certain macroalgae have antiviral activity (Johns *et al.*, 1979). By the year 2048, macroalgae is expected to emerge as alternative resource for fish oil, the traditional source of *n*-3 PUFA (Worm *et al.*, 2009). There is, therefore, an increasing interest in the use of edible macroalgae in the development of low-cost, highly nutritive diets for human and animal nutrition.

Fatty acid (FA) analysis has been increasingly gaining importance due to the realization of their beneficial applications in nutritional and health products. Recently, in biodiesel production, it has been shown that clean burn properties of the fuel are influenced by FA structural features including chain length and degree of unsaturation (Knothe, 2005). The author showed that, biodiesel consists of fatty acids esters, each of them affecting the properties of the fuel such as ultimately exhaust emissions, heat of combustion, cold flow, oxidative stability, viscosity, and lubricity. For instance, a long straight-chain hydrocarbon yield a high quality standard, while, a highly branched compound results poor ignition quality of the biodiesel. High quality of biofeul have been correlated with reduced nitrogen oxides (NOx) exhaust emissions, that has been reportedly increase with increasing unsaturation and decreasing chain length of the fatty acids.

Ulva spp. are the most abundant macroalaga in the coastal water of Alexandria yearly (Aleem, 1993) and represents unexplored natural resource with potential economic value for use in human and animal nutrition and as biofuel resource. Therefore, the purpose of the present study was to study the spatial and temporal variance in lipid content and its chemical characteristics in the green macroalgae Ulva.

# MATERIALS AND METHODS

## Study Area

Alexandria is the main harbor on the north coast of Egypt, extending about 32 Km along the coast of the Mediterranean Sea, at latitude 31,118836 ° N and longitude 29,551502°E. The macroalgal samples were collected from two stations namely Ras Al-Tin (A) and Al-Muntazah (B) along 18.6 Km distance, Alexandria coast (Fig. 1).

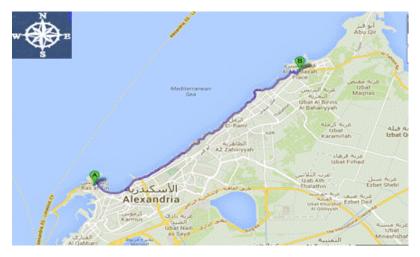


Fig. 1: Map illustrates the study stations (A and B) (from Google earth)

The station A was selected to represent a polluted region between Eastern Harbor, one of the main Egyptian fishery grounds with an area of 2.53 km<sup>2</sup> and an average depth of 6 m (El-Said and El-Sikaily, 2013) and the main Harbor of Alexandria (for export and import goods), while, station B represents unpolluted region (Al-Muntazah) as described in a previous study (El-Said and El-Sikaily, 2013). Station A can also be described to some extent as a sheltered zone while the station B is fully exposed and affected by the water current from the west to the east.

# Macroalgae

The fronds were tentatively identified as *Ulva fasciata* (Delile 1813). Nevertheless, identification was not made to species level because of the taxonomic complications and heteromorphism that are common in these genera (Villares and carballeira, 2003). Monthly samples of the green macroalgae Ulva sp. were collected from the submerged rocks and substrates at the assigned stations and washed with seawater to remove the extraneous matters as much as possible. Then the algal samples were immediately transported to the laboratory in an ice box containing frozen gel cold packs. On arrival, the macroalgae samples were gently scrubbed under running tap water to eliminate the other species of macroalgae as well as adhering epibiota (zooplankton and young bivalves) to the algal surface, sediments and detritus. and briefly rinsed with distilled water. All cleaned algal fronds were individually blotted on towel papers to remove excess water and then dried in the drying oven on 60±2 °C for 48h. Then, they were pulverized in a cereal grinder for 5 min and sieved, using a 100 mesh sieve, to obtain a fine and homogeneous powder that was stored in hermetic sealed plastic bags in the refrigerator until lipid determination and fatty acids analysis.

# **Total Lipid content determination**

The lipid content of the macroalgae samples was measured by solvent extraction method in a soxhlet system where petroleum ether was used as solvent (AOAC, 2000). The values are presented as percent of the dry weight (DW) of the samples.

# Fatty acid methyl esters (FAMEs) determination

Lipids were extracted with a chloroform-methanol mixture (2:1 v/v). The lipids in chloroform were dried over anhydrous sodium sulphate, and the solvent was removed by heating at 60°C under vacuum (AOAC, 2000). Fatty acid methyl esters (FAMEs) were prepared according to Vogel (1975). The analysis was performed in a gas liquid chromatograph equipped with dual flame ionization detector and dual channel recorder. FAMEs were separated through a coiled glass column (1.5×4 mm) packed with Diatomite (100-120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8°C min<sup>-1</sup> from 70°C to 190°C, then isothermally at 190°C for 25 min with nitrogen at 30 ml min<sup>-1</sup>. FAMEs were identified by comparing the retention times of experimental samples to those of known standards. The FAs values were represented as percent of the total FAs. The FAs analysis was conducted at the Regional Center for Food and Feed, Agricultural Research Center.

# **RESULTS AND DISCUSSION**

#### **Total lipid content:**

The lipid content of the green macroalgae *Ulva* sp. ranged between 6.0 and 11.6% DW with an annual average of 9.4±1.5% DW at the station A, and between 8.6 and 17.6% DW with an annual average of 12.2±2.7% DW at station B (Fig 2). However, no significant differences could be detected.

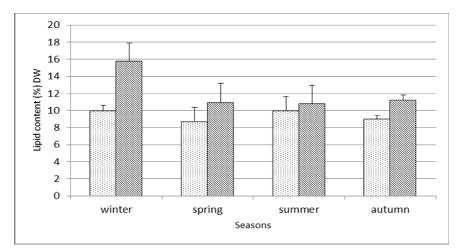


Fig. 2: Lipid content of *Ulva* sp.as (%) of dry weight (DW) collected from Station A (dotted columns) and station B (lined columns), bars are SD.

In general macroalgae are reported to have lipid content less than 5% of dry weight (Van Ginneken *et al.*, 2011; Khairy and El-Shafay, 2013; Kumari *et al.*, 2013a). However, some macroalgae at different locations showed higher lipid content. *Ulva lactuca* collected from Tunisia coast has lipid content of 7.9 % DW (Yaich *et al.*, 2011). Satpati and Pal (2011) reported a total lipid content of 12% DW in *Ulva rigida* collected from marine coastal region of Chilka Lake, India. These high lipid contents emphasize the approach for considering macroalgae as a promising resource for production of oil-based bioproducts.

The differences in the reported quantities of extracted lipid could have been due to many factors such as geographical and seasonal factors, or climate change as well as the development stage of the macroalgae as stated by (Ortiz *et al.*, 2006). Sánchez-Machado *et al.* (2004) recorded that, as the temperature increased, the lipid content in macroalgae decreased and remained almost stable until the end of the growing season. Similarly, the results of the present study showed a high content of lipid during winter and decrease in lipid contents from spring till autumn, particularly at station B.

The low lipid content at the station A can be explained by the pollution in that station which resulted in higher nitrogen and phosphorus contents as has been shown in a previous complementary study (Moustafa and Saeed, 2014). As the high lipid content can be used as indicator of nitrogen or phosphorus deficiency (Kumari *et al.*, 2013b).

#### Saturated fatty acids (SFAs) content.

As shown in Table (1), the SFAs contents ranged from 28.21 to 79.56% of TFAs content at station A, while ranged from 17.56 to 70.98% of TFAs content at station B, with the maximum content in autumn and the minimum content in spring at the both stations. The annual mean of the SFAs at station B, 54.7% of TFAs content, was higher than that at station A, 52.4% of TFAs content (Table 2). These results are consistent with the findings of Yaich *et al.*, (2011), who reported that Tunisian *Ulva lactuca* has SFAs in high percent (68.97%) of the TFAs. Also, Khairy and El-Shafay (2013) reported that the SFAs are predominant in green macroalgae *Ulva lactuca* collected from the north coast of Egypt, during three seasons, spring, summer and autumn with a range from 70 to 75% of the TFAs. A similar percent (70.01% of the TFAs) was reported also in *Ulva reticulata* by Shanmugam & Palpandi (2008). Kumari *et al.* (2010) reported that SFAs in 10 species of *Ulva* range from 51.7 to 63.7% of TFAs. However, lower content of SFAs (33.78% of TFAs) was reported in

Ulva lactuca collected from the coastal area of Northern Chile (Ortiz et al., 2006). Kumari et al. (2013a) studied that the FA compositions of 12 species belonging to the order *Ulvales* and reported that *Ulva lacutca* has the highest content of SFAs 59.9% of TFAs, while Ulva fasciata has the lowest content of SFAs 29.6 % of TFAs. In general, high contents of saturated fatty acids have been reported in warm water tropical macroalgae (Bhaskar et al., 2004).

In the present study, the main component of the SFAs was also the plamitic acid (C16:0), which ranged between 23.23 and 66.9% of TFAs content at station A, and ranged between 13.48 and 43.98% of TFAs content at station B, with the highest values in winter at station B (43.98%) and in autumn at station A (66.91%). Several studies reported palmitic acid as the main element of the TFAs (52.2%-59.4% of the TFAs content) in *Ulva lactuca* collected from Alexandria coast, Egypt (Khairy and El-Shafay, 2013), and from the Northern of Tunisia (Yaich et al., 2011), and in Ulva rigida collected from marine coastal region of Chilka Lake, India (Satpati and Pal, 2011). Shanmugam & Palpandi (2008) reported the dominance of C16:0 with a percent of 50.76% of the TFAs in *Ulva reticulata*.

Also a considerable percent of stearic acid (C18:0) was recorded in *Ulva* in the present work, ranging between 4.11 and 10.1% TFAs at station A, and from 2.73 to 24.67% TFAs at station B (Table, 1). Lower range (1.3-5.49% of the total FAs) of stearic was reported in *Ulva lacutca* by Khairy and El-Shafay (2013). These long chain SFAs (C16 and C18) are of utmost importance in biodiesel industry, since they yield high quality standard and short ignition time (Knothe 2005).

## **Content of Monounsaturated Fatty acids (MUFAs)**

The presented results in Table (1) revealed that the MUFAs, mainly omega-7 and omega-9, reached its maximum content in *Ulva*. sp. during spring with values of 33.39% TFAs at station A, and 30.45% TFAs at station B. Similar percent of MUFAs (36.66 % TFAs) was reported in *Ulva lactuca* by Ortiz et al. (2006). However, lower values of MUFAs were reported as maximum content of MUFAs in green macroalgae Ulva lactuca, 20.5% TFAs (Khairy and El-Shafay 2013). Also Kumari et al. (2013a) reported MUFAs contents within a range of 5.4-12.2% of TFAs in 12 species of *Ulva*.

The oleic acid accounted for the main component of MUFAs in the present work. It reached up to 30.7% of the TFAs content at station A and up to 28.69% of the TFAs content at station B. Similarly, Yaich et al. (2011) reported the FA oleic as the main component but at lower percent (15.9% of the TFAs) in Ulva lactuca macroalgae collected in Tunisia. Ortiz et al. (2006) also reported C18:1n-9cis as the most abundant fatty acid in the Chilean *Ulva lactuca*, accounted for  $27.42 \pm 2.60\%$  of the TFAs content. It is worthy to mention that *Ulva* samples, in the present study, showed wide range of these fatty acids among locations and over time, which indicates that the environmental factors as well as spatial factor exerted an important effect on the content of these FAs in *Ulva*. Thus, omega-9 and omega-6 fatty acids showed the highest content (29.11-30.7 and 37.1-49.58% of the TFAs content, respectively) in spring and the lowest content (7.4-12.89 and 1.05-3.72% of the TFAs content, respectively) in autumn.

The importance of MUFAs emerges from a study carried out by Huang et al. (2010) who, demonstrated that the n-6, n-7, n-9 fatty acids, such as palmitoleic acid, and oleic acid, as well as their esters exhibit strong antimicrobial activity against some microorganisms such as Streptococcus mutans, Candida albicans. Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum. and *Porphyromonas gingivalis.*, demonstrating some specificity for individual microbial species. Wang *et al.* (2013) also demonstrated that *Ulva lactuca* contain three MUFAs derivatives, of C18 and C16 FAs, which help in resisting many pathological conditions such as cardio-diseases and cancer.

# Content of Polyunsaturated Fatty Acids (PUFA):

The results in Table (1) revealed that the PUFAs maximum values were recorded in winter and spring (38.4% of the TFAs content) at station A, and in spring (52.1% of the TFAs content) at station B. In these occasions PUFAs showed higher values than MUFAs. During summer and autumn, MUFAs contents were higher than PUFAs at the both stations of study. Likewise, *Ulva lactuca* samples collected in July from north coast of Tunisia and in November from north coast of Chile and showed higher MUFAs contents than PUFAs content (Ortiz *et al.*, 2006 and Yaich *et al.*, 2011). Comparable PUFAs content (36.6% of the TFAs content) was reported in *Ulva rigida* by Satpati and Pal (2011). Nichols *et al.* (1998) showed that PUFAs may be very responsive to the environmental conditions changes, as they play important roles in algal physiology. It was found that temperature changes among seasons have a major effect on the FA composition of cell membranes (Phleger, 1991). El- Shoubaky *et al.* (2008) reported that low temperatures result in higher de-saturated fatty acids and PUFA content.

C18-PUFA acquire special importance in human nutrition and other vertebrates which are not able to synthesis them. Similarly, (Sánchez-Machado *et al.* (2004) and Van Ginneken *et al.* (2011) reported that C18-PUFA are nutritionally important because human and vertebrate cannot build up them within their bodies. However, fish can elongate and desaturate 18:2 n-6 fatty acid (Cowey, 1976), which presented in the investigated algae (Table, 1) in substantially high amounts (up to 37.1% at station A and 49.58% at station B). Satpati and Pal (2011) reported that among the unsaturated fatty acids 18:2 was highly observed in Indian *Ulva rigida*. Linoleic acid can be converted to arachidonic acid (C20:4, n-6) as reported by Simopoulos (2008).

In the present work, Ulva sp. samples contained linolenic (C18:3  $\omega$ -3), another essential fatty acid, in higher amounts (1.13-3.63 mg per g DW) than that determined in  $Ulva\ lactuca$  (0.13 mg per g DW) by Ortiz  $et\ al.$  (2006) and comparable to that (2.53 mg per g DW) reported in  $Ulva\ lactuca$  by Yaich  $et\ al.$  (2011).

On the other hand, PUFAs can be considered also as having an important ecological role. Alamsjah *et al.* (2005) have found that PUFAs of *U. fasciata* exhibit a potent algicidal activity against the red-tide phytoplankton *Heterosigma akashiwo*. As it has commercial importance in industries such as margarines industry. Hitherto, the margarine industry use both plants and seeds as a primary source of PUFAs (mainly C18:2 and C18:3) (Bemelmans *et al.*, 2002).

Table 1: Seasonal variation of fatty acids content in *Ulva* sp. samples collected from two stations along Alexandria coast, Egypt 2012.

		Castle station (A)			Muntazah station (B)			
Fatty acids	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
	%	%	%	%	%	%	%	%
C8:0	-	-	-	0.87	0.7	1	0.46	-
C10:0	-	-	-	-	1.13	1	0.86	-
C12:0	1.78	-	7.5	0.7	5.42	0.31	3.72	1.11
C14:0	1.34	-	1.33	1.63	6.98	0.12	5.66	1.43
C15:0	-	-	5.41	0.87	0.95	1	2	-
C15:1ω6	-	-	-	0.6	0.22	ı	-	-

C16:0	23.23	23.7	47.2	66.91	43.98	13.48	39.7	36.7
C16:1ω9	-	-	-	0.2	-	0.11	2.56	2.49
C16:1ω7	-	-	-	-	0.88	0.11	1	1.15
C17:0	_	-	-	0.2	0.29	0.2	1.22	2.39
C16:3ω4				0.2	0.27	0.2	1,22	4.68
C16:4ω3	-	-	-	-	-	-	3.12	-
C18:ISO	-	-	-	-	-	-	-	1.2
C18:0	4.11	4.51	10.1	7.32	9.38	2.73	6.97	24.67
C18:1ω9	29.13	30.7	15.6	7.2	26.89	28.69	16.89	9.95
C18:1ω7	1.55	1.64	2.7	0.26	-	1.14	3.39	4.03
C18:2ω6	36.81	37.1	8.28	0.45	1.8	49.58	4.7	3.72
C18:3ω6	-	-	-	-	-	-	0.36	-
C18:3ω3	1.56	1.3	-	-	-	2.11	3.57	2.1
C18:4ω3	-	-	-	-	-	-	3.3	0.94
C20:0	0.5	-	-	0.46	0.29	0.48	-	1.97
C20:1ω9	-	-	-	-	-	0.31	_	
C20:1ω7	-	-	0.74	2.73	0.21	_	_	
C20:1ω5	-	1.05	-	9.53	0.88	-	-	
C20:4ω3	-	-	-	-	-	0.2	-	
C20:5ω3	-	-	-	-	-	0.2	-	
C22:0	-	-	-	-	-	0.24	0.5	1.51
ni*	-	-	1.14	0.07	-	-	0.02	
Total	100.01	100	100	100	100	100.1	100	100.04
Saturated Fas	30.96	28.21	71.54	79.56	69.34	17.56	61.09	70.98
MUFAs*	30.68	33.39	19.04	20.52	29.08	30.45	23.84	17.62
PUFAs*	38.37	38.4	8.28	0.45	1.8	52.09	15.05	11.44
Total UFAs	69.05	71.79	27.32	20.97	30.880	82.54	38.89	29.06
PUFA ω6	36.81	37.1	8.28	0.45	1.8	49.58	5.06	3.72
PUFA ω3	1.56	1.3	0	0	0	2.51	9.99	3.04
Ratio ω6/ω3	23.596	28.538	-	-	-	19.753	0.507	1.224
PUFA/SFA	1.239	1.361	0.111	0.006	0.026	2.96	0.246	0.161
C16	23.23	23.7	47.2	67.11	44.86	13.79	46.38	45.02
C18	73.16	75.25	36.68	15.23	38.07	84.25	39.18	46.61
Total C16&C18	96.39	98.95	83.88	82.34	82.93	98.04	85.56	91.63

Data are presented as % of total fatty acids.

Table (2): Annual lipid content and Fatty acids compositions of macroalgae Ulva sp samples collected from two stations along Alexandria coast, Egypt 2012

Item		Ulva sp		
Fatty acids (%)	Castle (Station A)	Muntazah (Station B)		
Total Lipid content	9.4±1.5	12.2±2.7		
Saturated FAs	52.57	54.74		
MUFAs*	25.9075	25.2475		
PUFAs*	21.38	20.1		
Ratio MUFAs/PUFAs	1.21	1.26		
PUFA/SFA	0.679	0.848		

<sup>\*</sup> MUFAS: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; ni: not identified.

C16&C18	90.39	89.54

# Omega-6/Omega-3 ratio

Higher omega6/omega 3 ratios were recorded in the present work than those reported and nutritionally recommended in the literatures (Table, 3). However, in two occasions comparable values were recorded at station B, 0.507 and 1.224 during summer and autumn (Table, 1). Lower ratios are reported in many species of macroalgae as represented in Table (3). As recommended by the World Health Organization, this ratio should be less than 10 in order to prevent inflammatory, cardiovascular and nervous system disorders (Sánchez-Machado *et al.*, 2004).

Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio (i.e 15/1) promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6/omega-3 ratio, i. e. ~1) exert suppressive effects (Simopoulos, 2002).

Table 3: Ratios of n-6: n-3 fatty acids in various seaweed species, as reported in earlier studies.				
Seaweed species Ratio n-6: n-3 Reference				
Ulva fasciata	0.2	Kumari et al. (2013a)		

Seaweed species	Ratio n-6: n-3	Reference
Ulva fasciata	0.2	Kumari et al., (2013a)
Ulva lactuca	0.3	
Ulva erecta	0.5	
Ulva prolifera	0.6	
Ulva lactuca	0.17-0.29	Khairy and El-Shafay (2013)
Ulva lactuca	1.0	Van Ginneken et al., (2011)
Ulva tubulosa	1.83	Kumari <i>et al.</i> , (2010)
Ulva reticulata	1.84	
Ulva lactuca	3.03	
Ulva sp.	2.21	
Gracilaria debilis	18.8	
Gracilaria dura	27.7	
Gracilaria furgosoni	18.7	
Ulva lactuca	1.31	Ortiz et al., (2006)

Several studies attributed the variation of fatty acids in macroalgae to the environmental, and nutrients effects on quantitative fatty acid profiles, or genotypes with specific quantitative fatty acid profiles (Ortiz *et al.*, 2006; Van Ginneken *et al.*, 2011; Gosch *et al.*, 2012& Khairy and El-Shafay, 2013), thereby opening the possibility to optimize the fatty acid content and quality for human and animal nutrition or for oil production through specific culture conditions and selective breeding. Moreover, PUFAs and SFAs are reported in response with the area of cultivation. PUFAs content in *Ulva clathrata* was reported to vary from 33% to 13% of the TFAs in medium cultivation system (2.25 m²) and large system (8000m²), respectively, while SFAs showed an opposite trend (Peña-Rodríguez *et al.*, 2011).

# Content of C16 and C18 fatty acids content

In light of increasing energy demands, predicted fossil fuels shortage in the near future, and environmental concerns such as the production of greenhouse gas carbon dioxide, emerge the importance of searching for alternative renewable and cleaner energy sources.

Guldhe et al. (2014) reported that microalgae Scenedesmus sp., a well-known to be a potential feedstock for biodiesel production, its lipid content could be increased from 18.9-% DW to 29.65% DW by using different drying methods. Also, in optimal conditions, the microalgae Desmodesmus sp. produces fatty acids characterize with high percent of C16 and C18 (95% TFAs), SFAs, MUFAs and PUFAs account for 38.9%, 33.1% and 22.6% TFAs, respectively. This lipid quality makes it a good feedstock for biodiesel production (Ho et al., 2014). The analysis results of Ulva in the present work (Table, 1) are comparable with these quality paramters. The C16 and C18 fatty acids composed more than 82% TFAs at the both stations of the study, with the annual means of 90.4 and 89.5% TFAs at stations A and B, respectively (Table, 2). As the results in Table (1) showed that C18 FAs contents were higher than C16 FAs during spring at both stations and during winter at station A. These results are corroborated with the findings of Kumari et al. (2013a) who reported that chlorophyta spp. are characterized with higher C18 PUFAs in their lipid extraction.

The high content of C16 and C18, the main component of the biodiesel, in the lipid extraction of *Ulva* sp. makes this macroalgae as a potential feedstock for biodiesel production. Gosch et al. (2012) demonstrated macroalgae as a biomass source for oil-based bioproducts including biodiesel. Not only do several macroalgae have high total lipid content above 10% dry weight, but also these lipids are in the form of extractable fatty acids as the fatty acid C18:1, a predominant FA in most of them, is suitable as a biofuel feedstock. Suganya and Renganathan (2012) stated that *U. lactuca* biomass is a suitable source for the biodiesel production.

#### **CONCLUSION**

These results suggest that the green macroalgae *Ulva* sp. as a promising fatty acid resource for human and animal nutrition as well as for biodiesel production, due to the following features:

- 1-This *Ulva* sp. is a considerable resource for lipid even more than reported previously in literature for the other species of *Ulva*.
- 2-The main component of SFAs is pamitic acid (C16:0), fatty acids C18:1 (oleic) and C18:2 (linoleic acid) represent high percent of the TFAs in *Ulva* sp.
- 3-MUFAs and PUFAs constitute about half of the fatty acids content in this macroalgae, which are important in nutrition of human and animal, specially larval
- 4-Likewise microalgae, *Ulva* sp. is rich in PUFAs, accounting for 20% of TFAs, which are boon for their utilization in both fresh and dried form in human nutrition and aquaculture.
- 5- n-6 fatty acids contents are higher than  $\omega$ 3 fatty acids contents which resulted in high  $\omega 6/\omega 3$  ratio, however, the results indicate that under suitable environmental conditions, *Ulva* sp. may form a promising source to enhance food quality, prevent many diseases and maintain health.
- 6- C16 and C18, which are the main components for biofuel, ranged from 82.3 to 98.95% TFAs content, which suggests *Ulva* sp. as a good feedstock for biodiesel production.
- 7- It became evident that under suitable environmental conditions, the fatty acid content and quality of *Ulva* could be optimized for human and animal nutrition or for biodiesel production.

#### RECOMMENDATION

As, in the near future, it is expected that the current available PUFAs sources will be insufficient. That necessitates new sources and strategies have to be explored, among them expansion in macroalgae cultivating systems, such as integrated multitrophic Aquaculture system (IMTA) and off-shore systems. Also the increase demand for clean energy source can be met by increase the yield of macroalgae. However, further studies are needed to determine the influence of each environmental on the FAs profile of the green macroalga *Ulva* spp.

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#### REFERENCES

- Alamsjah MA, Hirao S, Ishibashi F, Fujita Y (2005). Isolation and structure determination of algicidal compounds from *Ulva fasciata*. Biosci Biotechnol Biochem 69: 2186-2192.
- Aleem AA (1993). The marine algae of Alexandria, Egypt, Univ. Alexandria, Alexandria, 139 pp.
- AOAC (2000). Official Methods of Analysis. 17<sup>th</sup> ed. Gaithersburg, Maryland, USA, AOAC International.
- Bemelmans WJE, Broer J, Feskens EJM, Smit AJ, Muskiet FAJ, Lefrandt JD, Bom VJJ, May JF, Meyboom-de Jong B (2002). Effect of increased intake of α-linolenic acid and group nutritional education on cardiovascular risk factors: The Mediterranean alpha-linolenic enriched Groningen dietary intervention (margarin) study. Am J Clin Nutr.,75:221–227.
- Bhaskar N, Kinami T, Miyashita K, Park S-B, Endo Y, Fujimoto K (2004). Occurrence of conjugated polyenoic fatty acids in seaweeds from the Indian Ocean. Z Naturforsch., 59:310–314.
- Cowey CB (1976). Use of synthetic diets and biochemical criteria in the assessment of nutrients of fish. Journal of Fish Research Board Canadian, 33:1040–1045.
- El-Said GF and El-Sikaily A (2013). Chemical composition of some seaweed from Mediterranean Sea coast, Egypt. Environ Monit Assess, 185:6089–6099.
- El-Shoubaky GA, Moustafa AMY, Salem EAE (2008). Comparative phytochemical investigation of beneficial essential Fatty Acids on a variety of marine seaweeds algae. Research J Phytochemistry 2:18–26.
- Gosch BJ, Magnusson M, Paul NA & DE NYS R (2012). Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts. *Global Change B iology Bioenergy* **4**, 919.930.
- Guldhe A, Singh B, Rawat I, Ramluckan K, Bux F (2014). Efficacy of drying and cell disruption techniques on lipid recovery from microalgae for biodiesel production. Fuel, 128: 46-52.
- Ho S-H, Chang J-S, Lai Y-Y, Chen C-N N (2014). Achieving high lipid productivity of a thermotolerant microalga *Desmodesmus* sp. F2 by optimizing environmental factors and nutrient conditions. *Bioresource Technology* 156:108-116.
- Huang CB, George B & Ebersole JL (2010). Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms Archives of Oral Biology, 55: 555-560.

- Ito K and Hori K (1989). Seaweed: chemical composition and potential uses. Food Review International, 5: 101-144.
- Johns RB, Nichols PD, & Perry G J (1979). Fatty acid composition of ten algae from Australian waters. Phytochemistry, 18:799–802.
- Khairy HM and El-Shafay SM (2013). Seasonal variations in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt. Oceanologia, 55(2):435–452.
- Knothe G (2005). Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters, Fuel Process. Technol. 86 1059–1070.
- Kumari P, Bijo AJ, Mantri VA, Reddy CRK& JHA B (2013a). Fatty acid profiling of tropical marine macroalgae: An analysis from chemotaxonomic and nutritional perspectives. *Phytochemistry*, 86, 44-56.
- Kumari P, Kumar M, Gupta V, Reddy CRK& Jha B (2010). Tropical marine macroalgae as potential sources of nutritionally important PUFAs. Food Chemistry, 120, 749-
- Kumari P, Kumar M, Reddy CRK, Jha B (2013b). Algal lipids, fatty acids and sterols. Functional Ingredients from Algae for Foods and Nutraceuticals, Pages 87-134.
- Mohamed S, Hashim SN, Rahman HA (2011). Seaweeds: a sustainable functional food for complementary and alternative therapy. Trends Food Sci. Technol., 23(2): 83-96.
- Moustafa YTA and Saeed S M (2014) Nutritional evaluation of green macroalgae, Ulva sp. and related water nutrients in the Southern Mediterranean Sea coast, Alexandria shore, Egypt. 4th Conference of Central Laboratory for Aquacult. Res., 35-55.
- Nichols PD, Virtue P, Mooney BD, Elliott NG, Yearsley GK, (1998). Seafood the good food: the oil (fat) content and composition of Australian commercial fishes, shellfishes and crustaceans, CSIRO Div. Mar. Res., Hobart, 100 pp.
- Ortiz J, Romero N, Robert P, Araya J, Lopez-Hernández J, Bozzo CE, Navarrete CE, Osorio A and Rios A (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulva lactuca and Durvillaea antarctica. Food Chemistry 99: 98-104.
- Peña-Rodriguez A, Mawhinney TP, Ricque-Marie D & Cruz-Suárez LE (2011). Chemical composition of cultivated seaweed Ulva clathrata (Roth) C. Agardh. Food chemistry, 129, 491-498.
- Phleger CF (1991). Biochemical aspects of buoyancy in fishes, [in:] Biochemistry and molecular biology of fishes. Vol. I. Phylogenetic and biochemical perspectives, P.W. Hochachka & T.P. Mommsen (eds.), Elsevier Sci., 209-247.
- Rameshkumar S, Ramakritinan CM, Eswaran K and Yokeshbabu M (2012). Proximate composition of some selected seaweeds from Palk bay and Gulf of Mannar, Tamilnadu, India. Asian Journal of Biomedical and Pharmaceutical Sciences, 3(16):1-5.
- Sánchez-Machado DI, López-Cervantes J, López-Hernández J, Paseiro-Losado P (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. Food Chem. 85: 439-444.
- Satpati GG, and Pal R (2011). Biochemical composition and lipid characterization of marine green alga *Ulva rigida*- a nutritional approach. J. Algal Biomass Utln., 2
- Shanmugam A, Palpandi C (2008). Biochemical composition and fatty acid profile of the green algae *Ulva reticulata*, Asian J. Biochem. 3 (1): 26–31.
- Simopoulos AP (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacothe-rapy 56 (8): 365–379.

- Simopoulos AP (2008). The importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and other chronic diseases. Exp. Biol. Med. 233:674–688.
- Suganya T and Renganathan S (2012). Optimization and kinetic studies on algal oil extraction from marine macroalgae Ulva lactuca *Bioresource Technology*, 107, 319-326.
- Van Ginneken VJT, Helsper JPFG, de-Visser W, van-Keulen H, Brandenburg WA (2011). Polyunsaturated fatty acids in various macroalgal species from north Atlantic and tropical seas, Lipids Health Dis., 10, 1–8.
- Villares R and Carballeira A (2003). Seasonal variation in the concentrations of nutrients in two green macroalgae and nutrient levels in sediments in the Rias Baixas (NW Spain) Estuarine, Coastal and Shelf Science, 58:887-900.
- Vogel AJ (1975). A textbook of practical organic chemistry, 3rd edn., Longman Group Ltd., London, 1200 pp.
- Wang R, Paul VJ & Luesch H (2013). Seaweed extracts and unsaturated fatty acid constituents from the green alga *Ulva lactuca* as activators of the cytoprotective Nrf2–ARE pathway. *Free Radical Biology and Medicine*, 57, 141-153.
- Worm B, Hilborn R, Baum JK, Branch TA, Collie JS, Costello C, Fogarty MJ, Fulton EA, Hutchings JA, Jennings S, Jensen OP, Lotze HK, Mace PM, McClanahan TR, Minto C, Palumbi SR, Parma AM, Ricard D, Rosenberg AA, Watson R, Zeller D (2009). Rebuilding Global Fisheries. Science.;325:578–585.
- Yaich H, Garna H, Besbes S, Paquot M, Blecker C and Attia H (2011). Chemical composition and functional properties of *Ulva lactuca* macroalgae collected in Tunisia. Food Chemistry, 128: 895–901.

#### **ARABIC SUMMARY**

كيمياء الدهون فى الطحلب الأخضر من نوع الأولفا: كمصدر محتمل للتطبيقات البيوتكنولوجيه من الساحل الجنوبي للبحر الأبيض المتوسط ـ شاطئ الأسكندرية ـ مصر

ياسر ثابت عبد المجيد مصطفى - أحمد مصطفى بطران مركز البحوث الزراعية، المعمل المركزى لبحوث الثروة السمكية بالعباسة - قسم الليمنولوجي

لقد تم في هذا البحث در اسة كل من القيمة الغذائية و التغيرات الكيميائية في المحتوى من الدهون في طحلب الأولفا الناتجة عن إختلاف مكان وموسم أخذ العينات حيث تم تجميع ١٢ عينة من الطحلب من كل من موقعي الدراسة - رأس التين (محطة ا) و المنتزة (محطة ب) على شاطئ الأسكندرية خلال الفترة من شهر يناير و حتىَّ شهر ديسمبرمن عام ٢٠١٢. ثم جمعت كل ثلاثة عينات لتمثل موسم واحد في كل محطة. و تم دراسة الخواص الكيميائية لمستخلص الدهون باستخدام جهاز (GLC)- و أظهرت النتائج إرتفاع المحتوى من الدهون في طحلب الأولفا (£9.5 ، ١.٢٠±١٢.٢ % من الوزّن الجأف- من المحطة (١) و المحطة (ب) على التوالي) - الأمر الذي يمكن تفسيره بإرتفاع مستوى التلوث في المحطة (١). أما الأحماض الدهنية فتشكلت أساسا من الأحماض الدهنية البالمتيك،الأوليك، اللينوليك. ولقد شكلت الأحماض الدهنية المشبعة نحو ٥٠% من الأحماض الدهنية الكلية. أما الأحماض أحادية عدم التشبع فشكلت نسبة ٦٦.١٧.٤ ٣٣% من الأحماض الدهنية الكلية. في حين أن الأحماض الدهنية عديدة عدم التشبع فتواجدت بنسبة عالية بلغت أقصاها في الشتاء و الربيع في كلا المحطتين- نحو ٤٨٠٤ و ٣٠٠٥ من الأحماض الدهنية الكلية في المحطة (١) و المحطة (ب) على التوالى. و يمكن إرجاع هذه النسبة العالية الى الترابط بين إنخفاض درجة الحرارة ودرجة عدم التشبع في الأحماض الدهنية. كما أن الأحماض الدهنية طويلة السلسلة (كربون ١٦ ، ١٨) - وهي المكون الأساسي للوقود الحيوي -فشكلت أكثر من ٨٢% من الأحماض الدهنية الكلية. و تشير هذه النتائج الى إمكانية إستخدام طحلب الأولفا في تغذية الإنسان و الحيوان وللمحافظة على الصحة حيث أنه غنى بالأحماض الدهنية أحادية عدم التشبع و كذا العديدة عدم التشبع كما يمكن إستخدام الطحلب كمصدر جيد لإنتاج الديزل الحيوى. كما أن المدى الواسع من الأحماض الدهنية يشير الى إمكانية تغير نسب الأحماض الدهندية من خلال التحكم في ظروف الإستزراع.