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# Influence of Extraction Methods on Lipid and Fatty Acids Content of Three Mediterranean Seaweeds

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**ABSTRACT**

Total lipid content of green (*Codium Fragile*), red (*Jania Rubens*), and brown (*Sargassum Vulgare*) were determined using two different methods. Total lipids content was ranged from 8.3% to 2.9% by dry weight and the highest total lipid content was observed in the *Codium Fragile*. Folch method was found to be more effective than Bligh-Dyer method. Fatty acids are determined by gas chromatography of their methyl esters (% of total FAMEs).

Generally, the predominant fatty acids (all results for Folch method) were saturated Palmitic acid (C16:0; 44.67% -34.90%), monounsaturated Oleic acid (C18:1 (n-9); 18.14% - 9.15%), polyunsaturated linoleic acid (C18:2 (n-6) 5.73% - 4.61%). α Linolenic acid (C18:3(n-3); 4.05% - 3.65%), Arachidonic acid (C20:4 (n-6); 7.62% - 7.42% ), and Ecosapentaenoic acid (C20:5 (n-3) 11.20% - 8.28%). The highest content of ω -3 fatty acids (15.21%) was determined in *Codium Fragile* using Bligh-Dyer method, while conversely, the highest content of ω -6 fatty acids (13.94%) Was observed in *Jania Rubens* using Folch method.

**KEY WORDS:** Seaweed, Algae, Lipid, Fatty Acid, Chemical Composition, Extraction Method.

## 1. INTRODUCTION

Macroalgae comprise a heterogeneous group of organisms mainly occurring in shallow costal and ecosystems (Guerry, 2009) they play crucial biological, environmental, and ecological roles in costal environmental (Carneiro, 2014). Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine. Today seaweeds are the raw material for many industrial productions like agar, align, and carrageenan but they continue to be wildly consumed as food in Asian countries. They are nutritionally valuable as fresh or dried vegetables, or as ingredients in a wide variety or prepared foods. In particular, seaweeds contain significant quantities of protein, lipids, minerals and vitamins (Manivannan, 2008).

Seaweed species and their products are used as renewable bio source in medical field and pharmaceutical industry: they show anti-viral (Soares, 2012), antioxidant (Indu and Seenivasan, 2013), anti-cancer (Park, 2013), anti-inflammatory (Vazguez, 2011), and anti-coagulant activities (Pushpamali, 2008). A clinical study expresses about the omega-3 PUFAs that it has important cardio-protective effect (Russo, 2009) and the reduction of cardiovascular disease (CVD) occur by taking of EPA and Docosahexaenoic acid (DHA) (Leaf, 2008). ARA, the active form of omega-6 PUFA, is found helpful to develop immune response, thrombosis and brain function. ARA and DHA are major constituents of cell membrane and play an important role in the structure of neurons in the central nervous system, where these are present at high concentrations (Bruno and Tassinari, 2011).

Fatty acid analysis has been overwhelmingly gaining importance due to biodiesel production, clean burn properties of the fuel are influenced by FA structural features including chain length and degree of unsaturation (Knothe, 2005). A precise quantification of FA can also use to predict the quality of biodiesel, which is reduced considerably with the increase in the amount of saturated FAs.

## 2. MATERIALS AND METHODS

**Sample Collection and Preparation:** Seaweed species were collected from the northeast Mediterranean coast of Syria (Figure.1) between April-June 2018. The seaweed samples were cleaned and washed with water to remove epiphytes sand and other extraneous matter and immediately transported to the laboratory where the algae were carefully rinsed with tap water several times to remove salts and shade dried then grounded into a fine powder. The lipid extraction efficiency of Bligh-Dyer and Folch was compared in the present study.

**Bligh-Dyer Method:** Macroalage samples were extracted first with chloroform: methanol (1:2 v/v) and the residues re extracted with small portions of chloroform: methanol (1:1 v/v) all the extracts were pooled together, flittered and mixed with an equal volume of chloroform and water (1:1 v/v) for phase separation. The low organic phases were collected and evaporated to dryness under nitrogen and total lipid contents were determined gravimetrically (Blight and Dyer, 1959).

**Folch Method:** Macroalage samples were extracted first with chloroform: methanol (2:1 v/v) and the residues re extracted with small portions of chloroform: methanol (1:1 v/v) all the extracts were pooled together, filtered and mixed with an equal volume of chloroform and water (1:1 v/v) for phase separation. The low organic phases were collected and evaporated to dryness under nitrogen and total lipid contents were determined gravimetrically (Folch, 1957).


**Figure.1. Map illustarte the Sampling site (from Google Earth)**

**Analysis of Fatty Acids Composition:** The FA composition of TL was determined by gas chromatography (GC) after conversion of fatty acyl croups in the lipid to their methyl esters. The acid methyl esters (FAME) were prepared as per method Prevot and Mordret (1976). After 10 minutes centrifugation (3500 rpm), the hexane layer was taken for GC analyses. These FAMEs were analyzed by chromatography (GC) (Shimadzu GC-14B). The GC was equipped with flame ionization detector (FID) and Omega was-320 fused silica capillary column (30m 0.32mm I.D*0.25µm film thickness). The detector, injector and column temperature were 260, 250 and $200^0$c. The carrier gas was helium with a flow of 50 Kpa. All the chemicals and solvents used were of analytical grade.

## 3. RESULTS AND DISCUSSION

The total lipid (TL) contents of analyzed samples were determined in extracts obtained by different methods (Figure.2). Evidently, extraction using a 1:2 mixture of methanol/chloroform (Folch method) resulted in higher contents of lipids among studied samples, ranging from 8.3% (*C. Fragile*) to 3.4% (*J. Rubens*) whereas extraction with 1:2 chlorofom/methanol (Bligh-Dyer method) was less effective in relation to lipid contents, that ranged from 7.5% (*C. Fragile*) to 2.9% (*J. Rubens*) for the same seaweed samples as in the previous analysis.

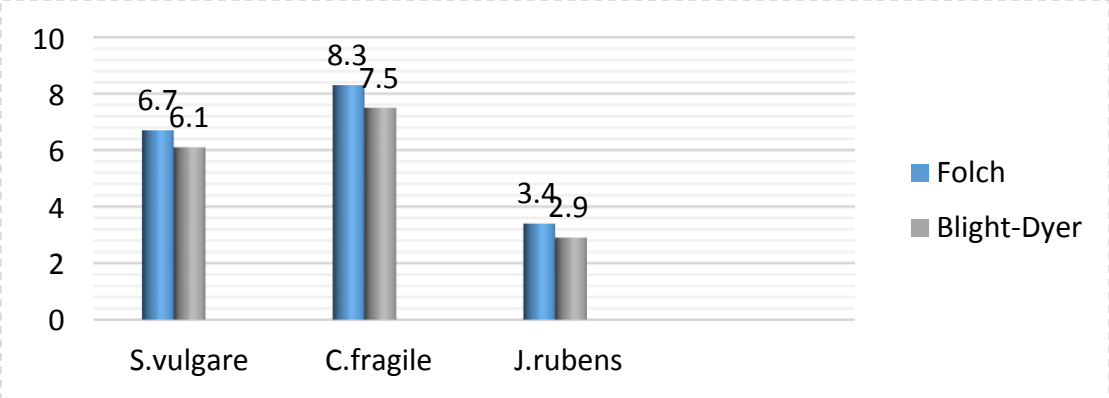


**Figure.2. Total lipid contents (%) of dry samples**

Reliable method for quantitative extraction of lipid and Fas are of paramount importance owing to their biochemical, physiological, clinical and nutritional application. The accuracy of different lipid extraction methods depends on the solubility of their constituent lipid classes in solvents employed and the nature of sample matrix as both could influence the extent of lipid extraction. According to Chrisite (1993), extraction solvents /mixtures should be polar enough to remove lipid their associating from ring cell constituents, but not too much polar that the solvents do not readily solubilize all the triacyl glycerols (TAGs) and other nonpolar lipids.

**Fatty Acid Composition:** The fatty acid contents of the three species in both method are presented in table.1, and the results are given in % of total FAMEs.

From the fatty acids identified, Palmitic (C16:0), Stearic (C18:0), Oleic (C18:1), Arachidonic (C20:4), and Eicosapentaenoic (C20:5) acids were the main fatty acids. These five fatty acids represents more than 78 % of all fatty acid.

The three seaweed species exhibited similar FA patterns but differed in their FA contents. All samples showed the highest proportion of SFA in their FAMEs distribution regardless of the method used. The highest

contents of SFAs obtained with Bligh-Dyer and Folch methods were established in the red seaweed *J. Rubens* (66.38% / 64.11%). Conversely, the green seaweed *C. Fragile* had the lowest contents of SFAs (53.817% / 53.012%). Surprisingly, The highest contents of SFAs obtained by Bligh-Dyer method. Palmitic acid (C16:0) was found to be the predominant SFA in all samples, present in a range from 45.98 % of total FAMEs (*C. Fragile*) to 36.48% (*J. Rubens*) for Bligh-Dyer method and from 44.67% (*C. Fragile*) to 34.9 % (*J. Rubens*) for Folch method. Generally, contents of Palmitic acid (C16:0) in green seaweed (*C. Fragile*) were determined as the highest from all samples with both methods. It was observed that the content of Palmitic acid (C16:0) in *J. Rubens* (34.9%/36.48%) was in keeping with the reported content of Palmitic acid (C16:0) in the red seaweed *J. Rubens* (34.22%) (Caf, 2019), but differed from the result reported for the same species in Portugal (44.44%) (Pereira, 2012). Similarly, in the brown seaweed *S. Vulgare*, same amount of Palmitic acid (C16:0) was measured (35.315%; 37.92%) in comparison with the published data (37.11%) (Caf, 2019).

Further, in *C. Fragile* lower amount of palmitic acid (C16:0) (44.67%; 45.98%) were found in comparison with the reported contents (49.14%) (Akgul, 2015), but similar amount was reported for the same seaweed (40.73%) (Pereira, 2012). Beside this, any seaweed had Pentadecanoic acid (C15:0).

**Table.1. Fatty acid profiles of studied seaweeds using Folch and Bligh-Dyer methods**

| Conventional Methods | | | | | | |
|---|---|---|---|---|---|---|
| | Folch method | | | Bligh-Dyer | | |
| **Fatty acids** | ***S. Vulgare*** | ***C. Fragile*** | ***J. Rubens*** | ***S. Vulgare*** | ***C. Fragile*** | ***J. Rubens*** |
| C8:0 | ND | ND | 0.812 | ND | ND | 0.928 |
| C10:0 | ND | 0.143 | 1.209 | ND | 0.136 | 1.289 |
| C11:0 | ND | 0.158 | 2.490 | ND | 0.155 | 0.662 |
| C12:0 | 1.553 | 1.314 | 2.032 | 1.355 | 1.116 | 2.012 |
| C13:0 | ND | 0.130 | 0.120 | ND | 0.122 | ND |
| C14:0 | 2.118 | ND | 3.465 | 2.189 | ND | 3.568 |
| C14:1 (n-5) | ND | 0.177 | 0.127 | ND | 0.015 | 0.131 |
| C16:0 | 35.315 | 44.672 | 34.908 | 37.922 | 45.982 | 36.487 |
| C16:1 (n-7) | 1.118 | 0.509 | 2.411 | 2.198 | 0.521 | 1.373 |
| C17:0 | ND | 0.103 | ND | ND | 0.072 | ND |
| C17:1 (n-7) | 2.758 | 0.058 | 0.148 | 2.958 | 0.041 | 0.055 |
| C18:0 | 18.962 | 6.034 | 18.912 | 18.121 | 6.122 | 20.012 |
| C18:1 (n-9) | 12.041 | 18.145 | 9.153 | 11.041 | 17.841 | 9.892 |
| C18:2 (n-6) | 4.611 | 5.028 | 5.731 | 4.172 | 5.891 | 6.954 |
| C18:3 (n-3) | 3.651 | 4.056 | ND | 3.549 | 4.779 | ND |
| C20:0 | 0.583 | 0.431 | 0.014 | 0.541 | 0.234 | 0.051 |
| C20:2 (n-6) | 0.478 | 0.082 | 0.561 | 0.370 | 0.101 | 0.024 |
| C20:3 (n-6) | 0.671 | 0.303 | 0.164 | 0.591 | 0.368 | 0.356 |
| C20:4 (n-6) | 7.622 | 7.421 | 7.491 | 6.919 | 6.083 | 6.091 |
| C20:5 (n-3) | 8.282 | 11.207 | 10.245 | 7.889 | 10.432 | 10.109 |
| C22:2 | 0.237 | ND | ND | 0.175 | ND | ND |
| ∑SFA | 58.531 | 53.012 | 64.110 | 60.128 | 53.817 | 66.382 |
| ∑MUFA | 15.917 | 18.889 | 11.691 | 16.197 | 18.525 | 10.078 |
| ∑PUFA | 25.552 | 28.097 | 24.192 | 23.665 | 27.654 | 23.534 |
| ∑UFA | 41.469 | 49.986 | 35.883 | 39.862 | 46.179 | 33.612 |
| ∑PUFA / ∑SFA | 0.436 | 0.530 | 0.377 | 0.393 | 0.513 | 0.354 |
| n6 / n3 | 1.141 | 0.840 | 1.361 | 1.068 | 0.818 | 1.042 |

In general, large difference were found in monounsaturated fatty acids (MUFAs) contents among the analyzed species. The highest contents of MUFAs were determined in *C. Fragile*, whilst the lowest contents were detected in *J. Rubens* regardless to method used. MUFAs were noticed in less amounts than SAFs contents ranged from 18.88 % (*C. Fragile*) to 11.69 % (*J. Rubens*) by Folch method and from 18.52 % (*C. Fragile*) to 10.078 % (*J. Rubens*) by Bligh-Dyer method. MUFAs contents obtained by Folch method were slightly higher than those obtained by Bligh-Dyer method. Oleic acid (C18:1) was found the most abundant MUFA in all samples by both methods. Content of Oleic acid ranged from 18.145% (*C. Fragile*) to 9.153 % (*J. Rubens*) using Folch method and from 17.84% (*C. Fragile*) to 9.98 % (*J. Rubens*) for Bligh-Dyer method. However, the results of Oleic acid C18:1 determined in almost samples are higher than the values reported in literature (Caf, 2019; Polat and Ozogul, 2013; Pereira, 2012; Akgul, 2015). The summed contents of oleic acid C18:1 in *C. Fragile* gave the highest amount (18.14% / 17.84%) of total FAMEs, like reported data (16.76%) (Akgul, 2015). The same situation was observed

in *S. Vulgare* where 12.04%/ 11.04% were determined, similar to a published 11.29% values for total FAMEs expressed as a sum of C18:1 (Caf, 2019). In the red seaweed higher contents of C18:1 (9.15% / 9.98%) were determined than reported in published data (8.38 %) (Caf, 2019) and (5.26%) (Polat and Ozogul, 2013).

PUFA contents ranged from 27.65% (*C. Fragile*) to 23.53% (*J. Rubens*) using Bligh-Dyer method, whereas Folch method were in range from 28.09% (*C. Fragile*) to 24.19% (*J. Rubens*) of total FAMEs. Predominantly, C20:5 was the most abundant fatty acid which varied from 11.20% (*C. Fragile*) to 8.28% (*S. Vulgare*) using Folch method and from 10.43% (*C. Fragile*) to 7.88% (*S. Vulgare*) using Bligh-Dyer method. Whilst the C20:4 contents were detected in similar proportion regardless to method used C20:4 content ranged from 7.62% (*S. Vulgare*) to 6.09% (*J. Rubens*). In the red seaweed higher contents of C20:5 (10.24% / 10.10%) were determined than reported in published data (4.36 %) (Caf, 2019) and (1.48%) (Pereira, 2012), but lower than reported data (12.18%) (Polat and Ozogul, 2013). α Linoleic acid C18:3 content did not exceed 4.77 % in all studied samples and was not observed in (*J. Rubens*). Moreover, small proportion of (C22:2) was only detected in *S. Vulgare* using both methods. Interestingly, the extraction of lipids by Bligh-Dyer method seemed to be less sufficient method for isolation of PUFAs with higher carbon numbers.

The PUFA / SFA ratio is a primary indicator used to evaluate the lipid quality, and its recommended minimum value is 0.45 by British Department of Health (1994). The PUFAs/SAFs fatty acids ratio could be used for a rapid evaluation of FA profiles of analyzed samples; the higher value of this ratio means more health benefits. Established low values of ratios in the products from brown *S. Vulgare* (0.43) and red seaweeds *J. Rubens* (0.37) agreed with higher presence of SAFs in their lipids, while the highest ratio was recorded in green seaweed *C. Fragile* (0.53).

The n-6PUFAs / n-3 PUFAs ratio should not exceed 10 according to WHO recommendation (Sanchez-Machado, 2004), while the European Nutritional Societies suggest this ratio not exceed 5 for the prevention of inflammatory, cardiovascular, and neurological disorders (Alles, 2014) all the examined species exhibited a favorable n-6 / n-3 ratio.

For biofuel production, seaweed with high proportion of saturated fatty acids are preferred as this leads to higher oxidative stability and higher ignition quality and produces an overall higher quality products (Hu, 2008; Knothe, 2008). The results from our study suggest that *Codium Fragile* and *Sargassum Vulgare* are the best biomass target for biodiesel production because of high total lipid and high proportion of SFAs.

## 4. CONCLUSION

Lipids and fatty acids (FA) composition of three Syrian macroalgae *Sargassum Vulgare*, *Codium Fragile*, and *Jania Rubens* were studied in two different methods. Total lipids varied within the investigated algal species and ranged from 8.3% to 2.9% on a dry weight basis. Fatty acids composition was analyzed by GC-FID. Saturated fatty acids (SFAs) were major components. Palmitic acid was the abundant fatty acid in all species tested. Eicosapentaenoic acid (C20:5 n-30) was found in significant quantities and accounted for more than 7% of total fatty acids in all samples. Interestingly, Folch method is more sufficient in extraction total lipid as well as MUFAs with higher carbon number than Bligh-Dyer method. All of the studied samples attested to the presence of health promoting nutrients such as PUFAs, especially essential ω-3 Fas, and this facts make *Codium Fragile* a useful food supplement, and n6/n3 ratio (1.36%-0.81%), which is within the recommendations by the World Health Organization to be lower than 10.

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