

Profiling of Omega 3 fatty acids from marine green algae *Ulva reticulata* and *Caulerpa racemosa*

Abirami S.¹, Murugesan S^{*1}, and Narender Sivaswamy S²

¹PG and Research Department of Botany, Division of Algal Biotechnology and Bionano Technology, Pachaiyappa's College, Chennai, Tamilnadu, India

²SynkroMax Biotech Pvt Ltd, Chennai, Tamilnadu, India.

*Correspondence Info:

Dr. S. Murugesan

Associate Professor,

Postgraduate and Research Department of Botany,

Division of Algal Biotechnology and Bionano Technology,

Pachaiyappa's College, Chennai 600 030, India,

E-mail: smurugesan5@gmail.com

Abstract

The applications of Omega-3 fatty acids for human health are rapidly expanding, which necessitates exploring alternative sources of fish. Single cell oils are now widely accepted in the market place and there is a growing awareness of the health benefits of PUFAs, such as gamma-linolenic acid (GLA), arachidonic acid (ARA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The high prevalence of chronic diseases worldwide indicates the requirement for alternative sources of omega 3 fatty acids. Increasing demand for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) containing fish oils is putting pressure on fish species and numbers. The present study impacts of the exploitation of the marine green algae *Ulva reticulata* and *Caulerpa racemosa* for their PUFA rich lipid profiling and screening for extraction methods of omega 3 fatty acids.

Keywords: Fatty acids, green algae, PUFA

1.Introduction

The marine ecosystem is one of the richest ecosystems among all. Marine algae, commonly known as seaweeds are one of the major potential living as well renewable resources of the oceans. Seaweed resources available around the world include more than 1000 species, from which only a hundred species are being commercially used. Seaweeds have been used as food, animal feeds, fertilizer and as sources of traditional medicine in many Asian civilizations since ancient times. Seaweeds are mainly utilized in phycocolloid industries, but many edible types of seaweed are rich in proteins, vitamins, minerals and trace metals. In order to fully exploit the nutritional value of seaweeds, several studies on the biochemical and nutritional composition of various seaweeds collected from different parts of the world have been conducted [1-8]. Indian market for omega-3 polyunsaturated fatty acid (PUFAs) ingredients is in a growth stage of its life cycle. Omega-3 fatty acids have been used in food fortification and dietary supplements for a long period in India. Consumer awareness of omega-3 health benefits and rising disposable income, especially in urban areas of the country, will lead the market growth in the future. It is estimated that the market will grow by a healthy rate of 11.4% CAGR from 2013 through 2020 in terms of volume, whereas in terms of value it is projected to touch US\$29.4 million by 2020 growing at a CAGR of 13%.

Macro algae has both pro- and anti-inflammatory compounds[9], the latter include sulphated polysaccharides (fucoindans) from brown seaweeds, alkaloids (Caulerpin I, II and III) from red and green seaweeds, polyunsaturated fatty acids (Docosahexaenoic acid: EDA, Eicosapentaenoic acid: EPA, Stearidonic acid: SA and Eicosatrienoic acid: ETA), carotenoids (fucoxanthin and astaxanthin), Pheophytin A and Vidalols A and B¹. The present study was done marine algae namely *Ulva reticulata* and *Caulerpa racemosa* for screening for the extraction of essential polyunsaturated fatty acids exclusively Omega 3 fatty acids, ALA (Alpha Linolenic acid), EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid).

2. Materials and Methods

2.1 Collection of seaweeds

The healthy seaweeds, *Ulva reticulata* and *Caulerpa racemosa* were collected from Mandapam. South East Coast of Tamilnadu, India. Seaweeds were rinsed with sea water and then with freshwater to remove epiphytes and adherents. These were air dried in shade at room temperature and extraction was carried out.

2.3 Extraction of total lipids

Soxhlet extraction was carried out to extract total lipids from macroalgae. One gram of dry algae was extracted from 4-5 h with 200 mL of the mixture of chloroform and methanol (MeOH) at 2:1 v/v in a soxhlet apparatus. The ratio was developed by Folch *et al.*, [10] based on the principle that the lipid extraction solvent must be adequately polar to remove lipids from their association with cell membranes and tissue constituents, but also not so polar that the solvent does not readily dissolve all triacylglycerols and other nonpolar lipids.

2.3. Total fatty acids extraction

One gram of crude lipid obtained were saponified by adding 35 mL of methanolic NaOH then kept at 100°C for 30 min and cooled immediately. Saponified lipid added with methanolic HCl solution at 80°C for 10 min and allowed for cooling. After methylation 50 mL of hexane was added to resultant solution to dissolve the esters [11]. Sufficient amount of NaCl aqueous solution or anhydrous sulphate were then added to allow the FAMES (fatty acid methyl esters) to float to the top of the flask, which was collected for further analysis.

2.4. Thin Layer chromatography

Thin layer chromatography was performed using Toluene and ethyl acetate (9:1) mixtures [12] as the mobile phase. Precoated silica plates were activated by keeping the plates at 90°C for 30 min and then air dried. Samples were spotted using capillary tubes along with the standards (EPA, DHA). After each run, the spots were visualized using iodine vapor [13]. Finally the presence of omega 3 fatty acids was confirmed by Gas Chromatography.

2.5. Fatty acid estimation by gas chromatography

Gas chromatography analyses were performed with HP 5890 Gas Chromatograph equipped with a split/splitless capillary (1:40) injector and a flame ionization detector. Analytical separation was achieved on an AT-1000 capillary column (25 m × 0.32 mm i.d.) with 0.2 µm film thickness (Alltech, Illinois, USA). Nitrogen [14] was used as carrier gas (with a constant flow rate of 1 mL/min). Temperature setting was controlled at injector 250°C, and detector 300°C. The oven temperature was held at 190°C. Quantifications of omega 3 fatty acid content in the unknown samples were calculated using EPA methyl ester (Eicosapentaenoic acid), DHA methyl ester (Docosahexaenoic acid) as external standards.

3. Results and Discussion

3.1 Omega 3 fatty acid composition of *Ulva reticulata* and *Caulerpa racemosa*

The results of the fatty acid composition analysis showed that *U. reticulata* and *C. racemosa* are very rich in n-3 fatty acids (Table.1). The fatty acid composition of each species were in the following ranges: saturated (SFA) 5.4 -32.4%, monounsaturated fatty acids (MUFAs) – 6.4-22% and polyunsaturated fatty acids (PUFAs) 0.3-24.5%. The percentages of SFA in *C. racemosa* to be lower than in *U. reticulata*. The highest MUFA content was obtained from *U. reticulata* (22%), whereas, *C. racemosa* had the lowest MUFA level. The highest level of PUFA (24.5%) was found in *U. reticulata*. The highest proportions of fatty acid in linolenic acid 24.5%, alphanolenic acid (C14:0, 16%), linolenic acid (C18:2n-6, 24.5%) found in *U. reticulata*. Whereas, the highest amount of morotic acid (1.5%). Eicosapentaenoic acid (EPA, C20:5n-3, 3.8%) and docosahexaenoic acid (DHA, C22:6 n-3, 2.14%) were found in *C. racemosa*. These results are in agreement with

previous studies on fatty acid distributions in macroalgae [15-16]. The recommended minimum value of the PUFA/SFA ratio is 0.45[17], which is higher in both species examined.

Table 1: Fatty acid profile of experimental algae (mg/g dry wt)

S. No	Fatty acids	<i>U.reticulata</i>	<i>C.racemosa</i>
1	Lauric acid	11.10 ± 0.10 ^f	-
2	Myristic acid	8.10 ± 0.10 ^e	-
3	Palmitic acid	12.31 ± 0.24 ^g	34.30 ± 0.10 ⁱ
4	Margaric acid	0.00 ± 0.00 ^a	10.75 ± 0.01 ^g
5	Stearic acid	5.37 ± 0.05 ^d	32.41 ± 0.01 ^h
6	Oleic acid	22.20 ± 0.10 ⁱ	6.40 ± 0.10 ^f
7	Linolenic acid	24.37 ± 0.11 ^j	2.36 ± 0.05 ^c
8	Alpha linolenic acid	16.10 ± 0.10 ^h	6.26 ± 0.05 ^e
9	Moroctic acid	0.00 ± 0.00 ^a	1.46 ± 0.05 ^a
10	Eicosapentaenoic acid	0.32 ± 0.03 ^b	3.84 ± 0.05 ^d
11	Docosahexanoic acid	0.63 ± 0.01 ^c	2.14 ± 0.01 ^b
F-Value		2200.0	13920.0
P-Value		0.000	0.000

Values are expressed as Mean ± SEM, n=3 as Anova test p<0.05% level.

Essential fatty acids (EFAs) have two or more double bonds in their carbon chain. There are two groups of EFAs, the n-6 and the n-3, defined by the position of the first double bond in the molecule starting from the carbon atom at the methyl end of the chain. EFAs have to be provided in the food since they cannot be synthesized within the body. Besides omega-3 fatty acids, omega-6 fatty acids also play a crucial role in brain function, as well as normal growth and development.

Humans and other mammals lack the enzymes necessary to insert a cis double bond at position w6 or w3 in fatty acids. Since PUFAs (especially EPA and DHA) are effectively synthesized only by aquatic organisms, humans can obtain these essential components by consuming marine/freshwater products. EPAs with five double bonds serve as precursors of prostaglandins and thromboxans via the cyclooxygenase system. As a consequence, dietary omega-3 PUFA helps to reduce the risk of heart disease, decreases low density lipoprotein (LDL) cholesterol, and prevents osteoarthritis and diabetes [18-19]. Seaweeds can be used as sources for n-3 fatty acids such as eicosapentaenoic acid (EPA). It has been reported that the lipid and fatty acid contents are influenced by the type of species, habitat conditions, season, genetic differences and locations [20-22]. Omega 3 fatty acids are essential nutrients which cannot be synthesized by mammals. Therefore, they must be incorporated through dietary sources. The three main omega 3 fatty acids include Alpha linolenic acid (ALA), Eicosapentaenoic acid (EPA), Docosohexanoic acid (DHA). The health beneficial eicosapentaenoic acid (EPA; C20:5n-3) was found in all seaweed species except *Gracilaria corticata*, with 5.4% of total fatty acids as the highest concentration found in *Colpomenia sinuosa*. The EPA content (0.3%) of *U. reticulata* observed in the present study was lower than that reported by Chakraborty and Santra [23] for the same species (2.90%). Docosahexanoic acid (DHA; C22:6n-3), another physiologically important long-chain PUFA, was found in low concentrations in all seaweeds (ranging from trace to 1.1%). *Ulva sp.*, *T. atomaria*, *C. spongiosus*, *Peyssonnelia sp.* and *B. secundiflora* possess the highest contents of n-3 PUFA, 1.07, 1.38, 1.19, 1.06 and 1.42 mg/g, respectively. Apart from *Ulva sp.*, in which ALA dominated, the n-3 profile of the remaining strains was essentially composed of EPA. DHA was not a major PUFA in any of the algae studied in this work [24].

The fatty acid composition of *C. racemosa* have shown dominating components, C16:0, C16:1 (n-7) and C18:3 (n-3), among saturated, monounsaturated and polyunsaturated fatty acids, respectively [25]. From the Table 1, the result shows that *U. reticulata* has shown highest yield of 6.2%, alpha linolenic acid per gram of dry algae, whereas *C. racemosa* shown 3.8% EPA and 2.14% DHA were observed high in *C. racemosa*, whereas, *U.reticulata* has shown the least quantity of EPA (0.3%) and DHA (0.63%) respectively. Terasaki *et al.*, [26] reported relatively high concentrations of AA (11.6–14.8%) and EPA (9.7–12.0%) in *Sargassum* spp., despite not detecting any DHA in their lipid extracts. Variation in the PUFA concentration and profile of different seaweeds has been reported to be mainly dependent on the local water temperature from which the seaweeds were collected with temperature species

containing higher levels of fatty acids with long chain and higher degree of unsaturation compared to tropical species [27].

Consumption of long-chain omega-3 PUFA are known to be beneficial to human health [28] and together with a low dietary *n-6/n-3* ratio, are fundamental for a diet with cardio-protective benefits. In the present study, the marine green algae, *C. racemosa* and *U. reticulata* contained the highest total *n-3* PUFA content with the lowest *n-6/n-3* ratio among the seaweeds examined.

4. Conclusion

The present study concluded that *C. racemosa* contains higher amount of omega 3-fatty acids than *U. reticulata*. These seaweeds could potentially be used, after processing, as a food. Further studies are needed on the nutritional and toxicological aspects of seaweed utilization as food and feed resources for human and animal consumption, respectively.

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Conflict of interest statement

We declare that we have no conflict of interest.

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