



Fatty acids (FAs) composition in marine macroalgae along west coast of India

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ARTICLE INFO	ABSTRACT
<p>Original Research Article Received on October 28, 2024 Revised on October 30, 2024 Accepted on November 25, 2024 Published on December 03, 2024</p> <p>Article Authors N. M. Pise, P. V. Chavan, S. V. Thite, C. J. Khilare</p> <p>Corresponding Author Email pisenavnath@gmail.com</p>	<p>This study focused on the nutritional analysis of three marine macroalgae from the Chlorophyta and Rhodophyta phyla, specifically examining their lipid and fatty acid compositions. The lipid content of <i>Gayralia oxysperma</i>, <i>Porphyra vietnamensis</i>, and <i>Hypnea musciformis</i> varied significantly among the species, ranging from 1.50% to 4.94% on a dry weight basis ($p < 0.01$). Notably, members of Chlorophyta exhibited higher levels of C18 polyunsaturated fatty acids (PUFAs) compared to C20 PUFAs, while Rhodophyta species had slightly higher levels of C20 PUFAs than C18 PUFAs. The major fatty acids found in Rhodophyta species were palmitic acid (PA), stearic acid (SA), oleic acid (OA), and arachidonic acid (AA), while <i>G. oxysperma</i> displayed high levels of linolenic acid (LA), alpha-linolenic acid (ALA), and docosahexaenoic acid (DHA). Overall, all studied species demonstrated a nutritionally beneficial n6/n3 ratio ranging from 0.39 to 2.48:1, indicating a balanced ratio of omega-6 to omega-3 fatty acids, which is important for human health.</p>
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Seaweeds, also known as marine macroalgae, are prominent and influential components of the marine ecosystem. Not only do they play a crucial role in marine primary productivity, but they also have the potential to provide valuable bioactive compounds with significant importance in the fields of pharmaceuticals, biomedicine, and nutraceuticals (Kumari *et al.*, 2010, Cardozo *et al.*, 2007). Some types of marine macroalgae can be directly consumed as a nutritious food source for humans, while others can be utilized as ingredients in medicinal and culinary preparations. The lipid content in macroalgae typically ranges from 1-5% of their dry weight, although certain species may have higher lipid contents. Generally, brown algae tend to contain higher levels of lipids compared to green and red algae. Within these lipids (Mishra *et al.*, 1993) reported that the Omega 3 and Omega 6 PUFAs are important components in the clinical applications.

Marine flora is known for its abundance of fatty acids, with arachidonic acid (AA, C20:4 and eicosapentaenoic acid (EPA, C20:5) being commonly found. Renaund *et al.* (1999) reported that the Linolenic acids are rich in the chlorophycean algae which serve as a pioneer of prosta-glandins and thromboxanes that play a role in inflammatory, immunological, and cardiovascular response. Additionally (World Health Organization (2003) reported that the Palmitic acid (PA), stearic acid (SA), and linolenic acid (LA) are used in the various industries like cosmetic and pharmaceutical as well as in the nutritional products also. Seaweeds, also known as marine algae, have long been recognized for their nutritional benefits, particularly as a source of essential fatty acids. However, they possess other remarkable abilities that contribute to their potential health benefits.

One of these abilities is their capacity to scavenge oxyradicals, which are reactive oxygen species (ROS) or free radicals that can cause oxidative damage to cells and tissues. Phloroglucinol and phenolics are bioactive compounds found in seaweeds that play a crucial role in combating oxidative stress. These compounds act as metal chelators, meaning they can bind to and neutralize metal ions, particularly transition metals like iron and copper. Metals can catalyze the production of harmful reactive oxygen species within cells, leading to oxidative damage. By chelating these metals, phloroglucinol and phenolics help prevent the formation of harmful ROS (Rodrigo and Bosco, 2006). Polyunsaturated fatty acids (PUFAs) play a vital role in maintaining human health and nutrition. They are essential fats that the human body cannot produce on its own and must be obtained through the diet. PUFAs are critical for various physiological functions, including brain development, immune system support, and maintaining healthy cell membranes. To better understand the lipid and fatty acid composition of marine macroalgae and their potential as a source of PUFAs, a comprehensive study was conducted. The study focused on analyzing the total lipid content and the specific fatty acid profiles of two different intertidal and one estuarine shallow water marine macroalgae species.

Materials and Methods

Chemicals

For the identification and quantification of FAs, following analytical grade standards were used: 37-component F.A.M.E. Mix C4-C24 (Supelco, USA), 7-hexadecenoic acid methyl ester (C16:1, n7) and stearidonic acid methyl ester (C18:4, n3) (Cayman chemicals, USA). The internal standard Undecanoic acid (C19:0) was purchased from Sigma. All the solvents used (such as chloroform, methanol, dichloromethane and hexane) were of HPLC grade and other reagents of analytical grade.

Algal Samples

Macroalgal samples were collected in triplicates during low tide periods during 2010-2012 from different sites along the Maharashtra and Goa coast, India. Fully grown thalli of each alga which were chosen for this study. A detailed list of macroalgal samples along with the description of their habitat and the geographical co-ordinates are presented in supplementary table 1. The samples were wrapped in wet tissue towels and transported to the laboratory in cool conditions in an ice box.

Table 1. Total lipid contents of selected macroalgal species

Seaweeds	Years	Lipid (% Dry Weight)*
<i>Gayralia oxysperma</i>	2021	4.94±0.04
	2022	3.94±0.71
	2023	4.20±0.63
	Average	4.36±0.51 ^{cd}
<i>Porphyra vietnamensis</i>	2021	4.01±0.27
	2022	3.70±0.46
	2023	3.72±0.07
	Average	3.81±0.17 ^{cbd}
<i>Hypnea musciformis</i>	2021	1.62±0.31
	2022	1.50±0.14
	2023	1.65±0.07
	Average	1.59±0.08 ^{bc}

Note: * Mean values of triplicate samples ± SD, ^{a-d} Values in a row without a common superscript are significantly different at P < 0.01.

They were immediately cleaned thoroughly with autoclaved seawater to remove the epiphytes and other undesired foreign matter from the surface of algal fronds. The cleaned fronds were then blotted with tissue paper; weighed 1.0 g of each sample was immediately frozen in liquid nitrogen in triplicate and stored at 4^oC, until the analysis commenced.

Lipid Extraction

Lipids were extracted by modified Bligh and Dyer method using chloroform methanol phosphate buffer (pH 7.5) (1/2/0.9), as modified by (Kumari *et al.*, 2010). One gram of dried ground algal powder was extracted with 15 ml of chloroform: methanol (1:2, v/v) and the residue were extracted thrice with small portions (10 ml) of chloroform: methanol (1:1, v/v). All the extracts were pooled, filtered and mixed with an equal volume of chloroform and water (1:1, v/v) for phase separation. The lower organic phase was collected and evaporated to dryness in a vacuum, and the total lipid content was determined gravimetrically.

Fatty Acid Methyl Esters Preparation and GC-MS Analysis

The gas chromatography-mass spectrometry (GC-MS) analysis of fatty acid methyl esters (FAMES) obtained from marine macroalgae samples. The GC-MS analysis is a common technique used to identify and quantify the fatty acids present in samples.

Transmethylation

To convert the fatty acids present in the lipid samples into FAMES, a process called transmethylation was carried out. This involved treating the lipid samples with 1% sodium hydroxide (NaOH) in methanol. The mixture was then heated at 55°C for 15 minutes. After this, 5% methanolic hydrochloric acid (HCl) was added to the mixture, and it was heated again at 55°C for 15 minutes. Finally 1 ml of milli-Q water was added to the mixture to stop the reaction.

Internal Standard

Undecanoic acid was used as an internal standard in the analysis. An internal standard is a known compound added to the sample to aid in quantification and to correct for variations during sample preparation and analysis.

Extraction

The FAMES obtained after transmethylation were extracted using hexane. Hexane is a commonly used solvent for extracting lipids and fatty acid derivatives.

GC-MS Instrument

The GC-MS analysis was performed using a Shimadzu QP-2010 gas chromatography-mass spectrometer. The instrument was equipped with an autosampler (AOC-5000) and a capillary column (RTX-5) for separation of the FAMES.

GC Conditions

The column temperature was initially set at 40°C for 3 minutes, and then it was ramped up at a rate of 5°C per minute until reaching 230°C. The column was maintained at 230°C for 40 minutes. Helium gas was used as the carrier gas with a flow rate of 1 ml/min. The injection volume was 0.2 microliters at a temperature of 240°C, and the split ratio was 1/30.

MS Conditions

The mass spectrometer was operated in electron compact mode with electron energy of 70 eV. The ion source temperature and interface temperature were both set at 200°C.

Identification and Quantification

The FAME peaks obtained from the GC-MS analysis were identified by comparing their retention times with those of a standard mixture of fatty acid methyl esters (F.A.M.E. Mix C4-C24; Sigma). The quantification of the fatty acids was performed by area normalization, where the peak areas of the identified FAMES were used to determine their relative abundance in the samples.

Indices

The unsaturation index (U.I.) is a calculated value used to assess the degree of unsaturation or the number of double bonds in fatty acids present in a sample. It provides valuable information about the overall unsaturation level of the lipid or fatty acid composition. The higher the U.I., the more double bonds are present in the fatty acids, indicating a higher degree of unsaturation (Poerschmann *et al.*, 2004).

Results and Discussion

Lipids

In the study, the total lipid contents of various algal species were analyzed, and the results are presented in the table 1. The data show that there are significant variations in lipid content among the different algal species investigated. The lipid content ranged from $1.50 \pm 0.14\%$ to $4.94 \pm 0.04\%$ DW. These variations in lipid content may be attributed to several factors, including the species' metabolic activities, growth conditions, and environmental factors. Understanding the lipid content of different algal species is crucial for various applications, including biofuel production, food and feed supplements, and the extraction of valuable compounds such as PUFAs. Additionally, variations in lipid content can also influence the potential commercial value and feasibility of utilizing these algae for different purposes. The results of the analysis indicate that among the algal species studied, the Chlorophyta member *G. oxysperma* exhibited the highest average lipid content of $4.36 \pm 0.51\%$ on a dry weight (DW) basis. On the other hand, the Rhodophyta members displayed relatively lower variations in lipid content, ranging from $1.50 \pm 0.14\%$ to $4.01 \pm 0.27\%$ DW. When compared to *G. oxysperma*, the Rhodophyta members showed lower lipid content.

These findings are consistent with previous reports on the total lipid content of various macroalgal species, which also indicated that the lipid content generally remains below 4% on a dry weight basis (Herbreteau, Coiffard, Derrien and De Roeck-Holtzhauer, 1997). This suggests that lipid content in macroalgae tends to be relatively moderate, with few species reaching higher lipid levels. The variations in lipid contents observed among different algal species can be attributed to several factors, including the species type and their physiological characteristics, as well as environmental factors. Different species of macroalgae have diverse metabolic pathways and growth strategies, which can influence their lipid synthesis and accumulation. Additionally, environmental factors such as light availability, temperature, nutrient availability, and salinity can also play a role in determining the lipid content of macroalgae. Chandini *et al.* (2008) also noted that variations in lipid content among macroalgae can be influenced by both species-specific factors and environmental conditions. These factors interact in complex ways to influence the overall lipid content of different algal species.

Overall, the findings of this study highlight the variability in lipid content among different algal species, with Chlorophyta member *G. oxysperma* showing the highest lipid content, and Rhodophyta members displaying relatively lower variations in lipid content. Understanding the lipid content of various macroalgae is important for evaluating their potential applications in different industries, such as biofuel production, nutraceuticals, and cosmetics, and for assessing their ecological roles and interactions in marine ecosystems. The lipid content in marine algae is of great importance as lipids serve as an energy reserve for these organisms and play a crucial role in various biological processes. Additionally, the lipid content of certain marine algae species makes them valuable for various industrial applications, including the production of biofuels, nutraceuticals, and functional food ingredients. In conclusion, the analysis of lipid contents in various algal species has revealed significant variations within and between species. The Chlorophyta member, *G. oxysperma*, exhibited the highest lipid content, while the Rhodophyceae members displayed relatively lower lipid content.

These findings contribute to our understanding of the lipid profiles of different algal species and their potential applications in various industries. Further research is required to explore the factors influencing lipid content in marine algae and to optimize their utilization for industrial purposes.

Chlorophyta (*G. oxysperma*)

Chlorophyta member *G. oxysperma*, belonging to the orders Ulvales, has been extensively studied in terms of its fatty acid (FA) compositions. Table 2 lists the FA compositions of *G. oxysperma*, which display a characteristic profile with high contents of C18 polyunsaturated fatty acids (PUFAs). Chlorophyta members, including *G. oxysperma*, are known to exhibit high levels of polyunsaturated fatty acids (PUFAs) (Khotimchenko, Vaskovsky and Titlyanova, 2002; Li, Fan, Han, and Lou, 2002). PUFAs are a type of fatty acid with multiple double bonds in their carbon chain, and they are particularly valuable for human health due to their essential nature and numerous health benefits. *G. oxysperma* also contains C20 PUFAs, such as arachidonic acid (C20:4n-6) and eicosapentaenoic acid (EPA) (C20:5n-3). However, their contents in *G. oxysperma* are reported to be significantly lower compared to those found in Rhodophyta species (a different family of algae).

The presence of significant levels of PUFAs, especially alpha-linolenic acid (ALA), linoleic acid (LA), and docosahexaenoic acid (DHA), is of particular importance in *G. oxysperma*. These PUFAs are known for their health-promoting properties, including cardiovascular benefits, anti-inflammatory effects, and support for brain and cognitive function. The relatively lower levels of C20 PUFAs, such as arachidonic acid and eicosapentaenoic acid, compared to *Rhodophyta* species may indicate some species-specific differences in lipid composition and metabolic pathways. It is worth noting that the specific content of PUFAs can vary depending on various factors, including environmental conditions, growth stage, and nutrient availability. The high levels of PUFAs in *G. oxysperma* make it a potential valuable source of these essential fatty acids for various applications in the pharmaceutical, nutraceutical, and functional food industries.

Table 2. Fatty acid composition of *G. oxysperma* given in means \pm SD (% of total FAME)

FAs	<i>Gayralia oxysperma</i>			
	2021	2022	2023	Average
C14:0	3.51 \pm 0.16	9.60 \pm 0.69	2.88 \pm 0.09	5.33 \pm 3.71 ^a
C15:0	1.21 \pm 0.08	0.96 \pm 0.07	0.98 \pm 0.06	1.05 \pm 0.13 ^a
C16:0	28.79 \pm 0.49	26.23 \pm 1.52	27.77 \pm 0.51	27.60 \pm 1.28 ^{cd}
C17:0	0.27 \pm 0.01	0.29 \pm 0.01b	0.31 \pm 0.01	0.29 \pm 0.02 ^{cd}
C18:0	4.28 \pm 0.25	3.96 \pm 0.14	9.39 \pm 0.61	5.88 \pm 3.04 ^b
C20:0	0.34 \pm 0.02	0.30 \pm 0.05	0.44 \pm 0.02	0.36 \pm 0.07 ^b
C22:0	0.69 \pm 0.04	0.74 \pm 0.05	0.59 \pm 0.03	0.67 \pm 0.07 ^a
C24:0	2.36 \pm 0.06	2.64 \pm 0.08	2.11 \pm 0.11	2.37 \pm 0.26 ^a
C16:1(n-7)	1.24 \pm 0.15	0.38 \pm 0.01	0.39 \pm 0.01	0.67 \pm 0.49 ^a
C18:1(n-9)	0.35 \pm 0.03	0.30 \pm 0.02	0.30 \pm 0.02	0.32 \pm 0.03 ^{cd}
C20:1(n-9)	n.d.	0.32 \pm 0.02	0.25 \pm 0.01	0.19 \pm 0.16 ^b
C22:1(n-9)	0.16 \pm 0.01	n.d.	n.d.	0.05 \pm 0.09 ^{ab}
C18:2(n-6)	14.82 \pm 1.22	13.05 \pm 1.15	15.49 \pm 1.54	14.45 \pm 1.25 ^{ab}
C20:3(n6)	0.29 \pm 0.01	0.25 \pm 0.00	0.14 \pm 0.01	0.23 \pm 0.07 ^a
C20:3(n3)	3.18 \pm 0.12	3.24 \pm 0.06	2.55 \pm 0.13	3.00 \pm 0.38 ^d
C20:4(n-6)	0.21 \pm 0.008	0.19 \pm 0.006	0.15 \pm 0.011	0.19 \pm 0.02 ^d
C20:5(n-3)	1.44 \pm 0.13	1.48 \pm 0.12	1.32 \pm 0.10	1.42 \pm 0.08 ^d
C22:6(n-3)	2.07 \pm 0.18	2.35 \pm 0.16	1.84 \pm 0.15	2.09 \pm 0.26 ^d
C18:3(n-3)	32.53 \pm 2.88	31.99 \pm 1.06	31.08 \pm 2.10	31.86 \pm 0.73 ^{cde}
C16:1(n-9)	2.29 \pm 0.36	2.17 \pm 0.16	1.99 \pm 0.15	2.15 \pm 0.14 ^d
C22:4(n-6)	n.d.	0.06 \pm 0.03	0.04 \pm 0.01	0.03 \pm 0.03 ^d
SFA	41.43 \pm 0.78	44.72 \pm 0.53	44.46 \pm 0.07	43.54 \pm 1.82
UFA	58.58 \pm 0.80	55.80 \pm 0.29	55.56 \pm 0.05	56.64 \pm 1.68 ^a
MUFA	4.04 \pm 0.55	3.17 \pm 0.16	2.93 \pm 0.18	3.38 \pm 0.58 ^{bc}
PUFA	54.54 \pm 1.29	52.63 \pm 0.33	52.63 \pm 0.20	53.26 \pm 1.10 ^d
C18PUFA	47.34 \pm 1.70	45.04 \pm 0.18	46.57 \pm 0.56	46.32 \pm 1.17 ^{de}
C20PUFA	5.13 \pm 0.27	5.17 \pm 0.07	4.18 \pm 0.23	4.83 \pm 0.5 ^{cd}
n3PUFA	39.22 \pm 2.46	39.07 \pm 1.00	36.79 \pm 1.76	38.36 \pm 1.35 ^{ab}
n6PUFA	15.32 \pm 1.23	13.55 \pm 1.14	15.83 \pm 1.56	14.90 \pm 1.19 ^{cde}
n3/n6	2.58 \pm 0.36	2.90 \pm 0.31	2.35 \pm 0.34	2.61 \pm 0.27 ^{ab}
n6/n3	0.39 \pm 0.06	0.35 \pm 0.04	0.43 \pm 0.06	0.39 \pm 0.04 ^d
PUFA/SFA	1.32 \pm 0.06	1.18 \pm 0.02	1.18 \pm 0.01	1.23 \pm 0.07 ^{ab}
UI	162.14 \pm 3.72	158.04 \pm 1.40	153.50 \pm 1.30	157.90 \pm 4.32 ^{cd}

Note: n.d.: Undetectable; ^{a-d} Values in a row without a common superscript are significantly different at P < 0.01.

Moreover, understanding the lipid composition of *G. oxysperma* and other algae can contribute to broader research on the ecological roles of these organisms in marine ecosystems and their potential impact on the food web and environmental processes. In the present study, *G. oxysperma* demonstrated higher PUFA contents (52.63-54.54% TFA) and lower saturated fatty acids (SFAs) (41.43-44.72% TFA) compared to other Ulvales species from the Gujarat coast (Kumari *et al.*, 2010). This contrasts with the higher PUFA and lower SFA contents reported in other Ulvales species.

It has been observed that cold water species generally have higher PUFA contents (Bhaskar, Hosokawa and Miyashita, 2004; Colombo *et al.*, 2006), while warm water macroalgal species have higher SFAs and oleic acid but lower PUFA contents (Khotimchenko, 2003). The members of the Ulvales order, including algae such as *Ulva lactuca*, are known to exhibit high levels of alpha-linolenic acid (ALA) as the characteristic polyunsaturated fatty acid (PUFA). This pattern is consistent with previous studies conducted by (Khotimchenko *et al.*, 2002 and Li *et al.*, 2002), which also reported elevated levels of ALA in Ulvales species.

Table 3. Fatty acid composition of *P. vietnamensis* given in means \pm SD (% of total FAME)

FAs	<i>Porphyra vietnamensis</i>			
	2021	2022	2023	Average
C14:0	14.29 \pm 4.11	18.93 \pm 0.35	13.57 \pm 0.48	15.60 \pm 2.13 ^c
C15:0	1.16 \pm 0.31	1.250 \pm 0.06	1.34 \pm 0.07	1.25 \pm 0.14 ^{bc}
C16:0	36.85 \pm 9.05	40.32 \pm 2.31	43.77 \pm 1.38	40.31 \pm 4.18 ^c
C17:0	0.29 \pm 0.05	0.31 \pm 0.04	0.11 \pm 0.006	0.24 \pm 0.02 ^{cd}
C18:0	6.65 \pm 1.70	8.58 \pm 0.42	6.93 \pm 0.37	7.39 \pm 0.75 ^{bcd}
C20:0	0.11 \pm 0.02	0.17 \pm 0.006	0.13 \pm 0.003	0.13 \pm 0.01 ^{cd}
C22:0	0.10 \pm 0.05	0.15 \pm 0.005	0.12 \pm 0.003	0.12 \pm 0.012
C24:0	0.09 \pm 0.03	0.06 \pm 0.004	0.13 \pm 0.007	0.09 \pm 0.013 ^b
C16:1(n-7)	1.11 \pm 0.30	1.48 \pm 0.05	1.97 \pm 0.14	1.52 \pm 0.126 ^c
C17:1(n-7)	0.18 \pm 0.05	0.19 \pm 0.007	0.19 \pm 0.013	0.19 \pm 0.026
C18:1(n-9) trans	2.81 \pm 0.67	2.97 \pm 0.15	3.38 \pm 0.06	3.05 \pm 0.329 ^d
C18:1(n-9)	8.53 \pm 1.90	9.12 \pm 1.01	10.67 \pm 0.19	9.44 \pm 0.85 ^{abc}
C20:1(n-9)	2.56 \pm 0.72	2.68 \pm 0.09	2.84 \pm 0.17	2.69 \pm 0.34 ^{bc}
C22:1(n-9)	12.25 \pm 14.3	3.76 \pm 0.35	3.96 \pm 0.23	6.65 \pm 8.09 ^b
C18:2(n-6)	5.09 \pm 6.84	1.56 \pm 0.06	1.73 \pm 0.11	2.79 \pm 3.90 ^{de}
C20:2(n-6)	0.80 \pm 0.20	0.81 \pm 0.03	0.84 \pm 0.03	0.81 \pm 0.09 ^{dc}
C20:3(n6)	0.92 \pm 0.25	1.04 \pm 0.05	1.13 \pm 0.01	1.03 \pm 0.12 ^c
C20:4(n-6)	1.98 \pm 0.56	2.01 \pm 0.06	2.21 \pm 0.09	2.07 \pm 0.28 ^b
C20:5(n-3)	1.90 \pm 0.54	2.13 \pm 0.07	2.22 \pm 0.16	2.08 \pm 0.24 ^a
C16:1(n-9)	2.25 \pm 0.63	2.41 \pm 0.09	2.67 \pm 0.12	2.44 \pm 0.30 ^a
SFA	59.57 \pm 15.27	69.80 \pm 1.78	33.86 \pm 0.45	54.41 \pm 8.20 ^{bc}
UFA	40.42 \pm 15.28	30.19 \pm 1.78	25.71 \pm 0.09	32.11 \pm 8.32 ^{dc}
MUFA	29.71 \pm 10.05	22.63 \pm 1.59	8.15 \pm 0.41	20.16 \pm 5.25 ^{cd}
PUFA	10.70 \pm 5.26	7.56 \pm 0.27	1.73 \pm 0.11	6.66 \pm 2.92 ^{bc}
C18PUFA	5.09 \pm 3.84	1.56 \pm 0.06	6.42 \pm 0.30	4.36 \pm 3.84 ^{bc}
C20PUFA	5.610 \pm 1.57	6.00 \pm 0.21	2.22 \pm 0.16	4.61 \pm 0.80 ^{bc}
n3PUFA	1.901 \pm 0.54	2.13 \pm 0.074	5.92 \pm 0.25	3.32 \pm 0.23 ^c
n6PUFA	8.80 \pm 5.81	5.42 \pm 0.20	0.37 \pm 0.01	4.86 \pm 3.29 ^c
n3/n6	0.30 \pm 0.20	0.39 \pm 0.004	2.66 \pm 0.09	1.12 \pm 0.10 ^{bc}
n6/n3	5.68 \pm 2.54	2.54 \pm 0.028	0.12 \pm 0.007	2.78 \pm 3.19 ^{cd}
PUFA/SFA	0.21 \pm 0.16	0.11 \pm 0.006	0.12 \pm 0.007	0.14 \pm 0.09 ^{cd}
UI	61.72 \pm 17.51	49.23 \pm 2.31	54.28 \pm 1.56	55.08 \pm 8.99 ^{bcd}

Note: n.d.: Undetectable; ^{a-d} Values in a row without a common superscript are significantly different at P < 0.01.

In the present study, the researchers observed a similar trend in the Ulvales species they analyzed, where the ALA content was found to be much higher than the linoleic acid (LA) content. Linoleic acid is another essential polyunsaturated fatty acid that is important for human health, and it is commonly found in plant-based oils. Indeed, the study by (Ortiz *et al.*, 2006) reported a higher LA content than ALA in *Ulva lactuca* from the Chile coast, and (Kumari *et al.*, 2010) found a similar trend in Ulvales species from the Gujarat coast in India. These findings indicate that the fatty acid composition of Ulvales algae may vary in different

geographical locations. The analysis of *G. oxysperma* revealed a relatively high content of polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA). However, when compared to other algae species, the DHA content in *G. oxysperma* was found to be relatively higher than *Caulerpa* sp. but lower than *Ulva* sp., as reported by (Kumari *et al.*, 2010). Additionally, the study highlights that the DHA content of Ulvales members can also differ based on their geographical location. For instance, *U. lactuca* from the Chile coast exhibited a DHA content of up to 0.8% of total fatty acids (TFA).

On the other hand, DHA was absent in Ulvaes members from the Bohai Sea (Li *et al.*, 2002) and California (Khotimchenko *et al.*, 2002). The study conducted by (Kumari *et al.*, 2010) compared the fatty acid (FA) composition of *G. oxysperma* and Ulvaes. The results demonstrated that *G. oxysperma* exhibited lower saturated fatty acid (SFA) contents, ranging from 41.43% to 44.72% of total fatty acids (TFA), compared to Ulvaes (p<0.01). However, *G. oxysperma* showed low levels of oleic acid, with only 0.30% to 0.35% of TFA, but higher levels of polyunsaturated fatty

acids (PUFA), accounting for 53.26% of TFA (p<0.01). This trend of higher C18 PUFA content and lower C20 PUFA levels is consistent with the findings of (Matanjun *et al.*, 2008) in their study on *Caulerpa lentillifera*, another member of the Chlorophyta family. The studies described a typical fatty acid profile for Chlorophyta algae, showing higher proportions of C18 PUFAs, including oleic acid, and relatively lower levels of C20 PUFAs. In terms of the n6/n3 ratio, the present study found that *G. oxysperma* ranged from 0.35 to 0.43.

Table 4. Fatty acid composition of *H. musciformis* given in means ± SD (% of total FAME)

FAs	<i>Hypnea musciformis</i>			
	2021	2022	2023	Average
C14:0	16.14±0.89	11.28±0.49	10.79±0.14	12.71±2.95 ^{bc}
C15:0	1.37±0.05	1.71±0.05	1.29±0.07	1.45±0.22 ^c
C16:0	55.30±1.16	56.51±0.77	54.96±2.71	55.59±0.81 ^{cbd}
C17:0	0.32±0.025	0.49±0.009	0.357±0.04	0.39±0.08 ^{ac}
C18:0	5.99±0.20	5.82±0.11	4.31±0.47	5.37±0.92 ^{ab}
C20:0	0.86±0.042	1.69±0.05	9.74±0.67	4.10±4.9 ^{ab}
C22:0	0.86±0.091	0.47±0.005	0.34±0.04	0.56±0.27 ^{dc}
C24:0	0.47±0.034	0.47±0.008	0.35±0.03	0.43±0.07 ^{cd}
C14:1	n.d.	0.16±0.003	0.36±0.04	0.17±0.18 ^{cd}
C15:1(n-5)	0.23±0.019	0.35±0.001	0.28±0.029	0.29±0.06 ^{cd}
C16:1(n-7)	5.33±0.17	5.75±0.04	5.35±0.67	5.48±0.23 ^{bc}
C17:1(n-7)	n.d.	0.35±0.003	0.36±0.04	0.23±0.20 ^c
C18:1(n-9) trans	3.18±0.03	3.99±0.15	3.67±0.13	3.61±0.41 ^{bc}
C18:1(n-9)	4.92±0.32	5.65±0.003	4.16±0.31	4.91±0.74 ^{bc}
C20:1(n-9)	0.73±0.02	0.35±0.004	0.50±0.06	0.53±0.19 ^c
C18:2(n-6)	0.81±0.01	1.23±0.031	0.98±0.044	1.01±0.21 ^b
C20:3(n6)	0.10±0.007	0.057±0.001	0.10±0.003	0.089±0.02
C20:4(n-6)	0.69±0.03	0.89±0.056	0.73±0.025	0.77±0.11 ^b
C20:5(n-3)	0.70±0.018	0.88±0.05	0.47±0.05	0.68±0.20 ^b
C18:4(n-3)	0.75±0.033	n.d.	n.d.	0.25±0.43
C16:1(n-9)	1.17±0.02	1.79±0.04	0.83±0.028	1.26±0.4 ^c
SFA	81.33±0.18	78.48±0.32	82.16±1.38	80.66±1.93 ^{cbd}
UFA	18.66±0.18	21.51±0.30	17.83±1.38	19.33±1.93 ^{ac}
MUFA	15.59±0.20	18.44±0.16	15.54±1.26	16.52±1.66 ^{bcd}
PUFA	3.07±0.03	3.07±0.142	2.29±0.122	2.81±0.45 ^c
C18PUFA	1.56±0.03	1.23±0.031	0.98±0.04	1.26±0.28 ^b
C20PUFA	1.51±0.007	1.838±0.11	1.304±0.079	1.55±0.26 ^b
n3PUFA	1.45±0.02	0.881±0.05	0.473±0.057	0.93±0.49 ^c
n6PUFA	1.61±0.017	2.189±0.087	1.817±0.067	1.87±0.29 ^{bd}
n3/n6	0.91±0.003	0.40±0.009	0.25±0.022	0.522±0.33 ^c
n6/n3	1.10±0.01	2.48±0.05	3.86±0.34	2.486±1.38 ^{cb}
PUFA/SFA	0.03±0.001	0.03±0.001	0.03±0.001	0.034±0.006 ^{cd}
UI	26.86±0.15	29.08±0.72	23.10±1.71	26.35±3.02 ^{bcd}

Note: n.d.: Undetectable; ^{a-d} Values in a row without a common superscript are significantly different at P < 0.01.

This ratio is considered important as it reflects the balance between omega-6 and omega-3 fatty acids, which play crucial roles in human health. A lower n6/n3 ratio is generally desired, as it indicates a higher proportion of omega-3 fatty acids, which have been associated with various health benefits. Moreover, the unsaturated index of *G. oxysperma* varied from 153.50 to 162.14, indicating a higher degree of total unsaturation compared to members of Rhodophyta and Chlorophyta ($p < 0.01$). The unsaturated index provides information on the overall level of unsaturation in the fatty acid composition and is often used to evaluate the nutritional quality of oils and fats. In conclusion, the study revealed that *G. oxysperma* had lower SFA contents compared to Ulvales, but exhibited low levels of oleic acid and higher PUFA contents. The C18 PUFA content was higher than C20 PUFA, similar to other Chlorophyta members. The n6/n3 ratio indicated a favorable balance of omega-6 to omega-3 fatty acids, and the unsaturated index reflected a higher degree of unsaturation compared to other algae groups. These findings contribute to our understanding of the fatty acid composition of *G. oxysperma* and its potential applications in various industries, including food and pharmaceuticals.

Rhodophyta (P. vietnamensis and H. musciformis)

In the present investigation, observed that these red algal species exhibited a distinct fatty acid pattern typical of *Rhodophyta* algae. The major fatty acids identified in these species included: Myristic acid (C14:0), Palmitic acid (C16:0), Stearic acid (C18:0), Oleic acid (C18:1 n-9), Docosenoic acid (C22:1 n-9). These fatty acids are commonly found in various algae species and contribute to their nutritional and health benefits. In addition to the identified fatty acids, the red algal species in the study also contained C20 polyunsaturated fatty acids (PUFAs), specifically arachidonic acid (C20:4n-6, AA) and eicosapentaenoic acid (C20:5n-3, EPA). These C20 PUFAs were found to be present at significantly higher levels compared to *G. oxysperma*, another algal species investigated in the study. The presence of C20 PUFAs, such as arachidonic acid and eicosapentaenoic acid, is particularly noteworthy as these fatty acids are known for their health-promoting properties. Both AA and EPA are essential omega-3 and omega-6 fatty acids that play

vital roles in human health, including supporting cardiovascular health, brain function, and anti-inflammatory responses. The significant differences in the fatty acid compositions between the red algal species investigated and *G. oxysperma* demonstrate the diversity in lipid profiles among different algae species. These variations may be attributed to genetic factors, species-specific characteristics, and environmental conditions in which the algae grow. Understanding the specific fatty acid profiles of different algae species is crucial for assessing their potential applications in various industries, including nutrition, pharmaceuticals, and cosmetics. The presence of high levels of essential PUFAs, such as AA and EPA, in the red algal species investigated by (Kumari *et al.*, 2010) highlights the nutritional value and potential health benefits of these algae for human consumption and commercial utilization.

These FAs accounted for a significant proportion (33.86–82.16%) of saturated fatty acids (SFA) in total fatty acids (TFA), while C18 PUFAs were present as minor components, ranging from 1.26% to 4.36% of TFA. The findings of the study conducted by (Kumari *et al.*, 2010) align with earlier research on red algae, which consistently reported C20 polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA) and eicosapentaenoic acid (EPA) as the dominant fraction of fatty acids in these algae. Several studies, including (Dawczynski *et al.*, 2007, Khotimchenko *et al.*, 2002, Khotimchenko and Gusarova, 2004, Li *et al.*, 2002 and Sanchez-Machado *et al.*, 2004), have previously documented similar findings. In the comparative analysis of PUFAs, specifically in the algal species *P. vietnamensis*, the researchers observed that the content of n-6 PUFAs was higher than that of n-3 PUFAs. The n-6/n-3 ratio ranged from 2.78:1, indicating a higher abundance of n-6 PUFAs relative to n-3 PUFAs in this particular algal species. Furthermore, the study revealed that AA was present in higher amounts than EPA in *P. vietnamensis*. Both AA and EPA are long-chain omega-3 and omega-6 fatty acids that are essential for human health. They play important roles in various physiological processes, including inflammation regulation, immune response, and cardiovascular health. The n-6/n-3 ratio is a significant indicator in evaluating the balance of these two types of essential fatty acids in the diet.

A balanced n-6/n-3 ratio, ideally close to 1:1, is considered beneficial for maintaining optimal health. In the case of *P. vietnamensis*, the n-6/ n-3 ratio was found to be relatively higher, suggesting a higher proportion of n-6 PUFAs in comparison to n-3 PUFAs. While both n-6 and n-3 PUFAs are essential for the body, an excessive intake of n-6 PUFAs in relation to n-3 PUFAs may have potential health implications, as an imbalanced ratio has been associated with increased inflammation and certain chronic diseases. However, the specific dietary impact of the observed n-6/n-3 ratio in *P. vietnamensis* would require further research and consideration. *H. musciformis* also exhibited higher levels of n-6 PUFAs compared to n-3 PUFAs, with an n6/n3 ratio of approximately 2.48, and the content of AA slightly higher than EPA. Interestingly, both studied species lacked docosahexaenoic acid (DHA). In the study conducted by (Colombo *et al.*, 2006), the researchers reported that the unsaturation index (U.I.) ranged from 23.10 to 61.72 in the algae samples they analyzed. This wide range of U.I. values indicates significant variations in the degree of unsaturation among the fatty acids in the samples.

The results of the study indicate that palmitic acid (C16:0) and myristic acid (C14:0) were the most abundant fatty acids (FAs) in both of the algae species investigated. Their content was significantly higher in Rhodophyta (*P. vietnamensis* and *H. musciformis*) compared to Chlorophyta (*G. oxysperma*). Oleic acid emerged as the major monounsaturated fatty acid (MUFA) in the studied species, with a range of 0.30-0.35% of TFA for *G. oxysperma*, 8.53-10.67% for *P. vietnamensis*, and 4.16-5.65% for *H. musciformis*. The contents of polyunsaturated fatty acids (PUFAs) within algal species have been the subject of numerous studies, with the aim of understanding their potential health benefits and commercial applications. The recent study on algal species revealed some interesting findings regarding the levels of polyunsaturated fatty acids (PUFAs) and the distribution of various fatty acids in these organisms. The study observed that the examined algal species contained noticeably higher levels of PUFAs compared to earlier studies conducted on related species. This increase in PUFA levels may have important implications for the nutritional and health benefits of these algae.

PUFAs, especially long-chain omega-3 and omega-6 fatty acids, are known for their positive effects on cardiovascular health, brain function, and inflammation regulation. Most of the algal species examined in the study contained approximately 90% trans fatty acids (TFA) as long-chain fatty acids with carbon chains ranging from C15 to C24. Trans fatty acids are unsaturated fatty acids with at least one double bond in the trans configuration. While some natural sources of trans fats can be found in small amounts, artificial trans fats, typically produced through hydrogenation of vegetable oils, are known to have negative health effects and should be avoided in the diet. The study reported that short-chain fatty acids with carbon chains ranging from C4 to C10 were not detected in the examined seaweed species. This absence may be attributed to the low amounts of these fatty acids in seaweeds, which were below the detection limit of the analysis. Short-chain fatty acids are typically found in small amounts and are less prevalent in marine organisms.

Fatty acids with carbon chains ranging from C11 to C14 were generally low in the examined algal species, varying between 1.87% of TFA in *C. veravalnensis* and 12.5% of TFA in *H. esperi*. The study on *H. musciformis* revealed C14:0 values ranging from 13.57% to 18.93% of TFA, which were higher than previously reported levels. This indicates that there may be variations in fatty acid composition even within the same species, possibly due to differences in environmental conditions or other factors. Among the important fatty acids identified, linoleic acid, cis-eicosadienoic acid, and arachidonic acid were found to represent predominant proportions of this fatty acid class.

Indeed, polyunsaturated fatty acids (PUFAs) have been extensively studied for their health benefits, and their role in the prevention of various chronic diseases is well-documented. As stated by the World Health Organization (WHO) in 2003, PUFAs, particularly omega-3 and omega-6 fatty acids, play significant roles in promoting overall health and reducing the risk of several diseases. Some of the key health benefits associated with PUFAs include: cardiovascular health, osteoarthritis, diabetes, brain health, inflammatory conditions, and immune function.

As such, incorporating a variety of dietary sources rich in PUFAs, such as fatty fish, algae-based products, flaxseeds, chia seeds, and walnuts, can be beneficial for overall health and disease prevention. However, individuals with specific health conditions or concerns should consult with healthcare professionals or registered dietitians for personalized dietary recommendations. Additionally, the green algae species examined in this study displayed interesting levels of alpha-linolenic acid (C18:3 ω 3) and stearic acid (C18:0), which are of great importance in the pharmaceutical and food industries. Stearic acid also finds utility in the production of candles, shampoos, soaps, plastics, and other cosmetic products. It can also serve as a hardener in candies when used in combination with simple sugar or corn syrup (WHO, 2003). The previous research conducted by (Banaimoon, 1992 and Fleurence *et al.*, 1994) highlighted the presence of appreciable amounts of essential fatty acids in different seaweed species. These essential fatty acids play crucial roles in various physiological functions and are vital for normal growth and development.

These fatty acids have been found to be beneficial in the prevention of cardiovascular diseases and other chronic ailments, including diabetes, hypertension, and autoimmune diseases. Despite macroalgae having low lipid contents (<4 g 100 g⁻¹ DW), their PUFA contents are equivalent to or even higher than those of terrestrial vegetables. The presence of important PUFAs, such as linoleic acid (LA), alpha-linolenic acid (ALA), gamma-linolenic acid (GLA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which have proven medical and nutraceutical applications, indicates their commercial significance in various industries. The fatty acid composition of marine macroalgae is of significant interest due to its nutritional value. Moreover, the presence of fatty acids can impact the applications of marine macroalgae in various industries, including food, nutraceuticals, and aquaculture and biofuel production.

Conclusion

The study's findings indicate that macroalgae offer a promising and sustainable source of important PUFAs with significant commercial

potential in various industries. The utilization of these marine algae can not only enhance the nutritional value of products but also contribute to improved human health and sustainable practices.

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References

- Banaimoon, S. A. (1992) Fatty acids in marine macroalgae from southern Yemen (Hadramout) including occurrence of eicosatetraenoic (20:4) and eicosapentaenoic (20:5) acids, *Botanica Marina*, **35**: 165-168.
- Bhaskar, N., Hosokawa, M., and Miyashita, K. (2004) Comparative evaluation of fatty acid composition of different Sargassum (Fucales, Phaeophyta) species harvested from temperate and tropical waters, *Journal of Aquatic Product Technology*, **3**: 53-70.
- Cardozo, K. H. M., Guaratini, T., Barros, M. P., Falcao, V. R., Tonon, A. P., Lopes, N. P., *et al.* (2007) Metabolites from algae with economic impact, *Comparative Biochemistry and Physiology, Part C*, **146**: 60-78.
- Carreau, J. P. and Dubacq, J. P. (1978) Adaptation of macroscale method to the microscale for fatty acid methyl transesterification of biological lipid extracts, *Journal of Chromatography*, **151**: 384-390.
- Chandini, S. K., Ganesan, P., Suresh, P. V. and Bhaskar, N. (2008) Seaweeds as source of nutritionally beneficial compounds – A review. *Journal of Food Science and Technology*, **45**(1): 1-13.
- Colombo, M. L., Rise, P., Giavarini, F., Sngelis, L. De., Galli, C., and Bolis, C. L. (2006) Macroalgae as sources of polyunsaturated fatty acids, *Plant Foods for Human Nutrition*, **61**: 67-72.
- Dawczynski, C., Schubert, R. and Jahreis, G. (2007) Amino acids, fatty acids and dietary fibre in edible seaweed products, *Food Chemistry*, **103**: 891-899.

- Fleurence, J., G. Gutbier, S. Maubeau and C. Leray (1994) Fatty acids from 11 marine macroalgae of the French Brittany coast, *J. Appl. Phycol.*, **6**: 527-530.
- Herbreteau, F., Coiffard, L. J. M., Derrien, A. and De Roeck-Holtzhauer, Y. (1997) The fatty acid composition of five species of macroalgae, *Botanica Marina*, **40**: 25-27.
- Khotimchenko, S. V. (2003) Fatty acids of species in the genus *Codium*, *Botanica Marina*, **46**: 456-460.
- Khotimchenko, S. V. and Gusarova, I. S. (2004) Red algae of Peter the Great Bay as a source of arachidonic and eicosapentaenoic acids, *Russian Journal of Marine Biology*, **30**(3): 183-187.
- Khotimchenko, S. V., Vaskovsky, V. E. and Titlyanova (2002) Fatty acids from the Pacific coast of North California, *Botanica Marina*, **45**: 17-22.
- Li, X., Fan, X., Han, L., & Lou, Q. (2002) Fatty acids of some algae from the Bohai Sea, *Phytochemistry*, **59**: 157-161.
- Matanjan, P., Mohamed, S., Mustapha, N. M. and Muhammad, K. (2008) Nutrient content of tropical edible seaweeds, *Euchema cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*, *Journal of Applied Phycology*, **21**: 75-80.
- Mishra, V. K., F. Temelli, B. Oraikul, P. Shacklock and J. S. Craigie (1993) Lipids of the red alga *Palmaria palmata*. *Bot. Mar.*, **36**: 169-174.
- Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernandez, J., Bozzo, C. *et al.* (2006) Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea Antarctica*, *Food Chemistry*, **99**: 98-104.
- Poerschmann, J., Spijkerman, E. and Langer, U. (2004) Fatty acid patterns in *Chlamydomonas* sp. as a marker for nutritional regimes and temperature under extremely acidic conditions, *Microbial Ecology*, **48**: 78-89.
- Kumari, Puja, Kumar, Manoj, Gupta, Vishal, Reddy, C. R. K. and Jha, Bhavanath (2010) Tropical marine macroalgae as potential sources of nutritionally important PUFAs, *Food Chemistry*, **120**: 749-757.
- Renaud, J., Axelson, D. and Birmaher, B. (1999) A risk benefit assessment of pharmacotherapies for clinical depression in children and adolescents, *Drug Safety*, **20**: 59-75.
- Rodrigo, R. and Bosco, C. (2006) Oxidative stress and protective effects of polyphenols: Comparative studies in human and rodent kidney: A review. *Comp. Biochem. Physiol.*, **142**: 317-327.
- Sanchez-Machado, D. I., Lopez-Cervantes, J., Lopez-Hernandez, J. and Paseiro-Losada, P. (2004) Fatty acids, total lipid, protein and ash contents of processed edible seaweeds, *Food Chemistry*, **85**: 439-444.
- WHO (2003) Diet, nutrition and the prevention of chronic diseases, WHO Technical Report Series 916, Report of a Joint WHO/ FAO Expert Consultation, World Health Organization, Geneva, Available at: http://whqlibdoc.who.int/trs/who_trs_916.pdf.