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The marine macro algae as potential source of nutritionally important polyunsaturated fatty acids

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ABSTRACT

The distribution of Poly unsaturated fatty acids exhibited a wide variation among the seven species of marine macroalgae. They were high in Phaeophyceae followed by Chlorophyceae and Rhodophyceae. The n - 3 fatty acids were 14.3% in Ulva fasciata which is the highest among the macroalgae analysed. The n – 6 fatty acids were more in Phaeophyceae than in Chlorophyceae and Rhodophyceae. The C18:2 and C18:3 were high with 7.8 and 12.9% respectively in U. fasciata. The C20:3 were relatively high in Padina tetrastromatica. The C20:4 and C20: 5 were high in Sargassum vulgare and S. tenerrimum. The n- 6/ n- 3 PUFAs ratio was 1.6 to 20.2 in Phaeophyceae, 1.28 to 1.52 in Rhodophyceae and 0.62 to 0.92 in Chlorophyceae which was lower than the WHO recommended value for diet.

Key words: Chaemotaxonomy, Chlorophyceae, marine macroalgae, nutrition, Phaeophyceae, polyunsaturated fatty acids, Rhodophyceae,

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INTRODUCTION

Fatty acid molecules have a variable length carbon chain with a methyl terminus and carboxylic acid head group. They can be categorised based on the degree of saturation of their carbon chains. Saturated fatty acids possess the maximal number of hydrogen atoms, while monounsaturated fatty acids and polyunsaturated fatty acids have one or two or more double bonds respectively. The polyunsaturated fatty acids can be further subdivided on the basis of location of first double bond relative to the methyl terminus of the chain. N- 3 and n -6 fatty acids are the two of the most biologically significant polyunsaturated fatty acid classes, and have their double bond on either of the third or sixth carbon from the chain terminus respectively. The final carbon in the fatty acid chain is also known as omega carbon, hence the common reference to these fatty acids as omega -3 or omega -6 polyunsaturated fatty acids. The long chain n-3 and n – 6 poly unsaturated fatty acids are synthesized from the essential fatty acids such as alpha - linoleic acid and linolenic acid respectively [1]. An essential fatty acid cannot be made by the body and must be obtained through the dietary sources. Because n – 3 and n -6 path ways compete with one another for enzyme activity, the ratio of n – 6 to n -3 polyunsaturated fatty acids is very important to human health. An ideal ratio is closer to 1:1 or 2:1. The marine macroalgae form a good, durable and virtually inexhaustible source for polyunsaturated with an n – 6 to n -3 fatty acids ratio of about 1.0 [2] The marine oil has been subjected to many studies and specific PUFA have interesting medical applications against disease [3]. Generally, the main n- 3 PUFAs exert arteriosclerosis, anti hypertension, anti inflammation, immune regulation effects etc.

Considering the indispensable importance of PUFAs in health and nutrition, seven different intertidal species of macroalgae of Visakhapatnam coast were analysed for their fatty acids. These primary producers form the feed for marine organisms and they are the important part of marine food chain. The fatty acid composition of these species can also be assessed for their chaemotaxonomic relationships in algal classification.

MATERIALS AND METHODS

Seven marine macroalgae namely *Ulva fasciata, Caulerpa sertularioides* belonging to Chlorophyceae, *Padina tetrastromatica, Sargassum tenerrimum, S. vulgare* belonging to Phaeophyceae, *Gracilaria corticata and Hypnea musciformis* belonging to Rhodophyceae were collected from the intertidal zone of the Visakhapatnam coast, east coast of India during the low tide period from November 2012. They were immediately brought to the laboratory, washed with fresh water and cleaned to remove all the extraneous matter. They were shade dried, powdered and used for fatty acid analysis.

About 10 g of the dried biomass of the sample was taken In 25 ml of 4% H_2SO_4 in methanol and refluxed for six hours. Methanol was evaporated partially and fatty acid methyl ester (FAME) was extracted with ethyl acetate and washed with water. The organic sample was evaporated from the extract, dried under vacuum and concentrated in the rotavap. The fatty acid composition of algal oil was analysed qualitatively using GC-MS and quantitatively using GC. The analysis was carried out on Agilent 6890N gas chromatograph connected to an agilent 5973 mass selective detector at 70 ev (m/z 50 -550:sources at 230 ◦C and quadruple at 150 ◦C) in the electron impact mode with a HP 5 capillary column (30m X 0.25 mm ID X 0.25 µm film thickness). The oven temperature was programmed for 2 minutes at 160 ◦C and raised to 300 ◦C at 5 ◦C/ minute and maintained for 20 minutes at 300 ◦C, and the split ratio was 50:1. The structural assignments were based on interpretation of mass spectrometry fragmentation and confirmed by comparision of retention times as well as fragmentation pattern of authentic compounds. GC analysis was performed on HP 6850 gas chromatograph equipped with FID detector and DB – 225 capillary columns (30 mm X 0.25 mm ID X 0.25 µm film thickness). The injector and detector temperatures were maintained at 300 and 325 ◦C respectively. The oven temperature was programmed for 2 minutes at 160 ◦C and raised to 300 ◦C at 5 ◦C/ minute and maintained for 20 minutes at 3000 ◦C. The carrier gas nitrogen was used at flow rate of 1.5 ml/minute. The injection volume was 1 µL, with a split ratio of 50:1. The identification of individual fatty acids is based on retention time of authentic fatty acid.

RESULTS AND DISCUSSION

The fatty acids belonging to seven species of marine macro algae are presented in Table 1. The polyunsaturated fatty acids were high in Phaeophyceae with variation from 39.8 to 47%. It was followed by Chlorophyceae with a variation of 26.8 to 37.5%. These PUFAs are relatively low with a variation of 11.2 to 26.9% in Rhodophyceae than the other two classes of macroalgae. The highest number of fatty acids were nineteen found in Phaeophyceae members. They were eighteen to nineteen in Rhodophyceae and seventeen to eighteen in Chlorophyceae. The number of PUFAs also varied with respect to their class. They were nine in Phaeophyceae, eight and nine in Rhodophyceae and seven and eight in Chlorophyceae.

The distribution of PUFAs varied in the seven species of macroalgae. The C18:2 linoleic acid was high in *U. fasciata* with 7.8%. It was 6.4% in *P. tetrastromatica*, 5.9% in *S. vulgare* and 4.5% in *S.tenerrimum*. In the remaining three species it was less than these levels. The C18:3 linolenic acid was 12.9% in *U. fasciata*, 5.8% in *C. sertularioides*. It was 4.9% in *S. tenerrimum* and 4.5% in *P.tetrastromatica*. In the remaining three species it was below these levels. The C20:2 ecosadienoic acid was observed as a trace with 0.2% in *P. tetrastromatica* and it was not detected in the remaining six species. The C20:3 DHLA was 1.6% in *P. tetrastromatica* and 1.0% in *S. tenerrimum*. In the remaining five species it was less than 1%. The C20:4 arachidonic acid was 8.3% in *S. tenerrimum*, 8.2% in S. vulgare, 5.9% in *P. tetrastromatica* and 4.1% in *H. musciformis*. It was below these levels in the remaining five species. The C20:5 eicosapentaenoic acid was 2.15% in *S.tenerrimum*, 1.8% in *S. vulgare* and 1.3% in *H. musciformis* 1.2% in *C. sertularioides*. It was less than 1% in *U. fasciata* and *P. tetrastromatica* and nil in *G. corticata*. The brown macroalgae were rich in unsaturated fatty acids. The C18 PUFAs were high in Phaeophyceae and Chlorophyceae than in Rhodophyceae. The C20 PUFAs were high in Phaeophyceae and Rhodophyceae than in Chlorophyceae. These findings agree with the earlier reports [4-8]. The *Sargassum* species were with higher amounts of n-3 PUFAs when compared to that of n -6 PUFAs [9]was not found in the present study. The sum of n-3 fatty acids was the highest in *U. fasciata* with 14.3%. It was 7% in *C. sertularioides*, 8% in

S. tenerrimum, 6.4% in *S. vulgare*, 6.8% *in P. tetrastromatica*. In *H. musciformis* and in *G. corticata* it was low with 4.6% and 1.4% respectively. The n-6 fatty acids were high with a variation of 12.5 to 14 % in Phaeophyceae. It was 6.5 to 8.9% in Chlorophyceae and 1.8 to 7.0% in Rhodophyceae. The n-6/ n-3 PUFAs ratio was 1.6 to 2.2 in Phaeophyceae, 1.28 to 1.52 in Rhodophyceae and 0.62 to 0.92 in Chlorophyceae. The lowest ratio of 0.62 was present in *U. fasciata* and the highest of 2020 was in *S. vulgare*. The original value of n -3/ n- 6 ratio was one for balance intake of both polyunsaturated n- 6 and n- 3 fatty acids. The high dietary intake of n -6 fatty acids from vegetable oils causes detrimental turnover of the balance ratio to n – 3 fatty acids. The ratio is 50 in Europe and USA, 12 in Japan which is compared to one in Green land Eskimos due to their higher consumption of Fish fatty acids [10]. The WHO recommended n -6/ n-3 ratio was lower than 10. The n – 6/ n- 3 ratio of marine macroalgae reported in the present study was below this limit. Low n-6/ n- 3 fatty acid ratios in Chlorophyta members were also reported [11]. The n -3 fatty acids increase the endothelium derived relaxing factor which in turn facilitates relaxation of large arteries and vessels was reported [12].

The total saturated fatty acids among the seven species varied from 53 to 89%. Higher content of saturated fatty acids occurred in Rhodophyceae with a variation of 73 to 89%. In Chlorophyceae, the variation was 62 to 73% and in Phaeophyceae, it varied from 53 to 60%. The composition of saturated fatty acids reveals interesting results from a chaemotaxonomic point of view [12]. This study revealed that the C16:0 palmitic acid appeared to be the most abundant fatty acid, irrespective of species. Its content was significantly high in Rhodophyceae with a variation of 57.77 to 87.7%. In Chlorophyceae, it was 47.1 to 56.7% and in Phaeophyceae it was 40.4 to 45.5%. The brown and red algae contain C20:4 arachidonic acid and C20:5 eicosapentaenoic acid as major components. Similar pattern of fatty acids distribution was also reported earlier [13-14, 11, 15].

Table 1. Variations in fattyacids in seven species of macroalgae (% dry weight)

Fatty acid	U.fasciata	C.sertula-rioides	S.vulgare	S.tenerri-mum	P.tetrastr-omatica	G.corticata	H.muscif-ormis
C12:0	1.0	0.8	0.6	0.4	0.4	1.1	1.6
C14:0	1.8	2.6	7.9	6.5	7.1	2.8	6.7
C15:0	0.4	0.7	0.6	0.6	0.8	0..7	1.2
C16;0	47.1	56.7	45.5	45.4	40.4	78.7	57.7
C16:1 n7	4.8	4.1	5.4	6.1	10.8	4.0	5.2
C16:2	1.9	2.0	0.6	0.5	0.8	1.0	0.8
C17:0	5.7	1.7	0.3	0.6	0.3	0.4	0.4
C18:0	1.2	1.8	2.5	1.7	2.0	2.6	1.8
C18;1 n9	9.5	3.1	12.1	13.2	16.8	3.4	10.5
C18:2 n6	7.8	4.5	5.9	4.5	6.4	0.9	2.9
C18:3 n3	12.9	5.8	3.8	4.9	4.5	0.8	2.7
C20:0	0.3	2.8	0.5	0.4	0.5	0.2	0.3
C20:1 n9	-	1.1	1.1	1.1	-	0.5	0.6
C20:2 n6	-	-	-	-	0.2	-	-
C20:3 n3	0.7	-	0.8	1.0	1.6	0.6	0.6
C20:4 n6	1.1	2.0	8.2	8.3	5.9	0.9	4.1
C20:5 n3	0.7	1.2	1.8	2.1	0.7	-	1.3
C22:0	2.6	1.8	0.9	1.1	0.3	0.3	0.9
C22:1 n9	-	1.9	0.7	0.8	0.2	0.1	0.3
C24:0	0.4	5.5	0.8	0.8	0.2	0.4	0.3
Σ SFA	62.0	73.0	60.0	58.0	53	89.0	73.0
Σ PUFA	37.5	26.8	39.8	42.0	47.1	11.2	26.9
Σn3PUFA	14.3	7.0	6.4	8.0	6.8	1.4	4.6
Σn6PUFA	8.9	6.5	14.1	12.8	12.5	1.8	7.0
n6/n3 PUFA	0.62	0.92	2.20	1.6	1.83	1.28	1.52

\- *Fatty acid not detected*

CONCLUSION

The results obtained reveal that the Phaeophyceae members are the good sources of PUFAs. The n- 3 PUFAs were high in *U fasciata* and n – 6 PUFAs in *Sargassum* species. The availability of important PUFAs such as linoleic acid, a – linolenic acid, gamma linolenic acid, arachidonic acid and eicosapentaenoic acid with proven biomedical and neutraceutical applications indicate their potential utilization in preparation of low fat food. It is concluded that it is highly recommended to use seaweed in a diet to decrease the n-6/ n-3 ratio. The fatty acids can also be used to unravel chaemotaxonomic relationships among the species of macroalgae.

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