IIT DELHI INDUSTRY DAY 2019

Sustainable Environment

Physicochemical, micronutrient and biochemical properties of dried macroalgae and microalgae

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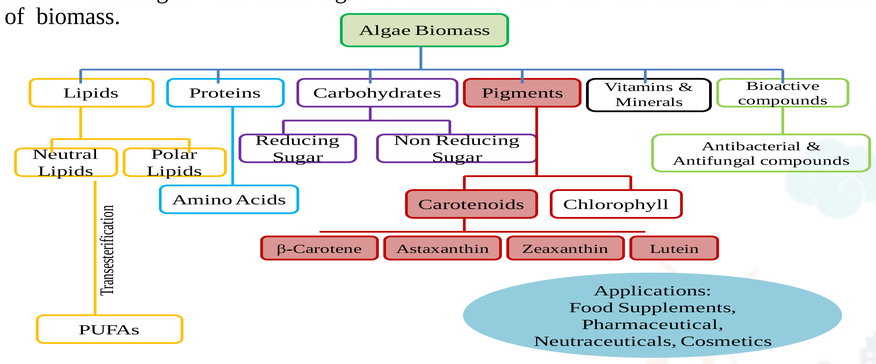
Abstract

Proximate value, mineral composition, fatty acids, protein is evaluated in green seaweeds (*Halimeda gracilis*), brown seaweeds (*Turbanaria conoides*, *Padina tetrostromatica*), red seaweeds (*Kappaphycus alverizi*, *Gelidiella ascerosa*, *Gracilaria verrucosa*) and P6 consortium microalgae. Results shows that seaweeds are rich in carbohydrate, protein and minerals but low in lipid content. All macroalgae species were high in ash (18-83%), carbohydrate (25-35%), protein (23-71%) on dry weight basis. Lipids were extracted using organic solvent extraction and Supercritical CO_2 extraction and result varies from 0.1-1.0%. The mineral content marginally among the species were in order of Na> K> Ca> Mg. Macroalgae species was found to be rich in polyunsaturated fatty acids (PUFAs) which include Palmitoleic acid, Oleic acid, Linoleic acid, Stearic acid, Eicosapentanoic acid (EPA), Arachidonic acid (AA) and Gamma-Linoleic acid (GLA). The studied species were rich in carotenoids, phenolic compounds, DPPH free radicals, therefore, they can be used as potential source of health food in human diets and may be of use in food industry.

Introduction

➢Selection of microalgae and macroalgae to extract PUFAs and carotenoids and determine physiochemical analysis of biomass.



➢Extraction of PUFAs and carotenoids from microalgae and macroalgae using different organic solvents and Supercritical carbon dioxide at optimum temperature and pressure

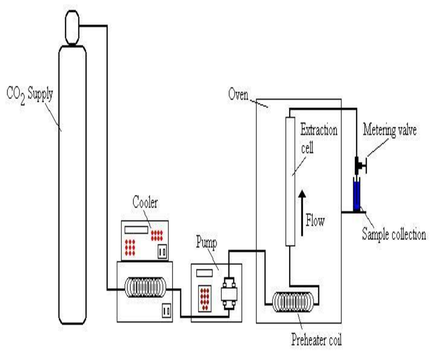
➢Characterization using Gas chromatography Mass spectroscopy and High Performance Liquid Chromatography

➢Application of extracted carotenoid in nutraceutical, cosmeceuticals and pharmaceuticals

Materials and Methods

➢Microalgae is grown and harvested on lab itself. Macroalgae are collected from different regions of Tamil Nadu, India (Pamban, 9.2651033, 79.2124088; Ervadi, 9.1992638, 78.7214408; Cabana coral leaf, 9.2838490, 79.1705430; Mandapam, 9.2769782, 79.1665524)

➢Extraction of PUFAs and carotenoids from selected microalgae and macroalgae using different organic solvents and Supercritical carbon dioxide at 40°C and 350 bars for 4-6 hours.



➢ Identification of PUFAs extracted from microalgae and macroalgae were done using Gas chromatography

➢Quantification of PUFAs using different standards were done using GC-MS

➢The separation, identification and quantification of carotenoids was made by HPLC where C18 column was used. The mobile phase (A) consisted of methanol-acetonitrile-water (84:14:2, v/v/v) and mobile phase (B) consisted of methylene chloride (100%).

➢ A standard curve was made and used for identification and quantification of carotenoids extract.

➢Protein was be isolated from supercritical by-products using TCA method and Kjeldahl method. Also pretreatment was done by ultra sonication and homogenization method.

Conclusions

➢ Major PUFAs present in microalgae and seaweeds

Soxhlet (Area%)				Supercritical CO_2 (Area %)			
Compound (% Area)	Chloroform : Methanol (1:1)	Hexane	Ethanol	SFE (150bar) 40°C	SFE (350 bar) 40°C	SFE (150bar) 50°C	SFE (350 bar) 50°C
Palmitic acid (16)	3.23	14.85	17.01	5.06	6.99	12.84	15.14
Palmitoleic acid (16:1/ω-7)	12.17	37.66	30.5	21.09	28.36	8.63	12.90
Stearic acid (18)	0.50		1.04	5.01	3.11	5.88	3.74
Oleic Acid (18:1)	3.53	7.28	13.72	30.28	24.89	17.98	23.34
Linoleic acid (18:2/ ω-6)		0.42	0.82	20.06		4.84	4.36

Compound (% Area)	*Kappaphycus Alvarezi*		*Halimeda gracilis*		*Turbanaria conoides*	
	Hexane	Ethanol	Hexane	Ethanol	Hexane	Ethanol
Myristic acid	7.51	4.13	13.48	6.13	1.50	3.90
Pentadecanoic acid	0.30	0.30	0.20	1.97	0.47	0.77
Palmitic Acid	19.23	35.01	21.12	25.36	14.59	22.6
Palmitoleic acid	10.16	13.01	2.44	6.04	2.56	3.47
Oleic acid	5.55	2.91	7.16	12.86	9.1	11.6
Linoleic acid	2.57	7.55	0.77	1.94	2.51	3.64
Stearic acid	5.86	6.44	8.42	4.02	4.12	2.75
Eicosapentanoic acid (EPA)	2.93	1.89	0.13	0.36	1.01	1.93
Arachidonic acid (AA)	3.6	2.57	2.04	3.11	4.82	6.88
γ-linoliec acid (GLA)	0.27	-	-	0.25	0.18	0.32
Cyclopentaneundecanoic acid	7.94	12.82	-	-	-	-

➢ Protein in microalgae and macroalgae were determined using lowry method and found high content of protein in microalgae (128 mg/g) and in macroalgae it varies from 0.4-50 mg/g by ultarsonication and homogenization method.

➢Total carotenoid was chemically extracted and it varies from 0.08-1.12 g/g

Industrial Significance

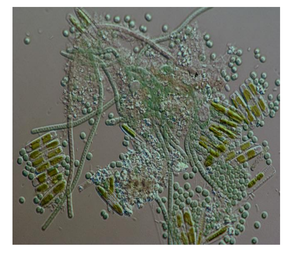
- This research work will help to develop a more economical and green process for extraction of PUFAs, proteins and carotenoids from microalgae and macroalgae
- Utilization of Supercritical CO_2 as extraction method will decrease the overall cost of the process.
- Microalgae is widely exploited as Nutraceutical, Cosmeceuticals, Pharmaceuticals and as animal and aquaculture feed.
- Public interest in recent times has focused on natural products with health-promoting properties as alternatives to conventional drugs.

Technology Readiness Level

High value product can be formulated using platform molecules present in microalgae and macroalgae such as proteins, carbohydrates and carotenoids which can be beneficial to human beings and animals. Also, these compounds reported to posses antimicrobial, anti-inflammatory, antioxidant, anticancer, antifungal, antiviral activities which can be used to prevent certain diseases.

Result

➢ Cultivation and harvesting of microalgae (*Chlorella pyrenoidosa* and *Phormidium* consortia) in Applied Microbiology Lab. IIT Delhi.



➢ Macroalgae collected from Tamil Nadu, India

Kappaphycus alvarezii

Gelidiella acerosa

Gracilaria verrucosa

Halimeda gracilis

Turbanari conoides

Padina tetrastromatica

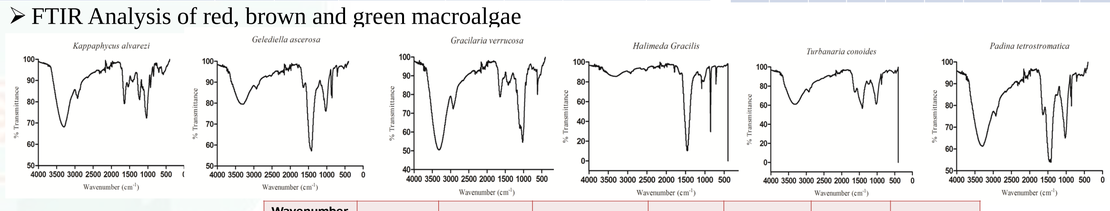
➢Physiochemical analysis of microalgae and macroalgae

Sample	C	H	N	Protein (Factor=5)	S	O	Moisture	Ash
Kappaphycus alvarezii	28.3	2.5	2.5	12.5	6.9	59.8	6.31	24.65
Gelidiella acerosa							8.45	29.2
Gracilaria verrucosa	35.3	5.9	2.3	11.5	5.6	39.4	8.91	18.67
Halimeda gracilis	17.1	1.1	1.9	9.5	-	70.4	1.41	83
Turbanari conoides	26.7	4.1	2.3	11.5	-	55.4	9.00	26.38
Padina tetrastromatica	25.7	3.9	2.4	12	-	56	8.50	42
P6 (*Phormidium* and *Chlorella pyrenoidosa* consortium)	36.7	5.7	4.9	30.6	-	51.5	8.6	14.2

➢ Mineral composition in algae

	Na	Mg	Al	K	Ca	Fe	Zn	As	Pb	Sr
KA	29.1	5.9	0.2	88.7	0.6	0.5	0.1	0.01	0.004	0.05
GA	2.04	2.2	0.7	0.3	2.2	0.5	0.3	0.007	0.004	0.1
GV	8.6	6.8	1.3	36.1	0.8	1.1	0.03	0.007	0.003	0.06
HG	2.45	2.12	2.9	0.58	46.9	1.9	0.08	0.004	0.003	4.77
TC	7.43	7.02	3.69	12.3	6.8	3.1	0.08	0.04	0.004	1.56
PT	4.66	8.78	2.92	4.46	10.4	2.72	0.11	0.01	0.004	1.42
P6	15.5	5.27	0.8	6.49	3.78	1.0	0.7	0.002	0.01	0.3

➢ FTIR Analysis of red, brown and green macroalgae

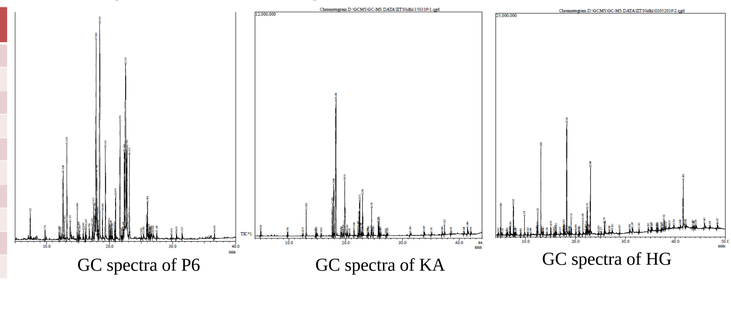


Wavenumber (cm^{-1})	3300-3200	1440-1330	1648-1638	1390-1380	1085-1010	995-985	850-550
Group	N-H	O-H	C=C	C-H	C-O	C=C	C-Cl
Compound class	Amine compounds	Carboxylic acid, Alcohol	Alkene (monosustituted)	Aldehyde	Primary alcohol	Alkene	Halo compounds

➢Extraction of lipids using organic solvents and Supercritical CO_2

Sample	Solvent extraction			SCO_2 (40°C/250bar
	Macroalgae			
	Hexane	Ethanol	Water	
Kappaphycus alvarezii	0.56	2.39	-	0.12
Gelidiella acerosa	0.34	1.1	-	0.13
Gracilaria verrucosa	0.48	3.43	18.14	0.44
Halimeda gracilis	0.24	0.51	0.52	0.1
Turbanari conoides	0.43	1.63	-	0.79
Padina tetrastromatica	0.27	1.8	15.92	0.14
	Microalgae			
P6	1.84	17.98	5.49	0.98

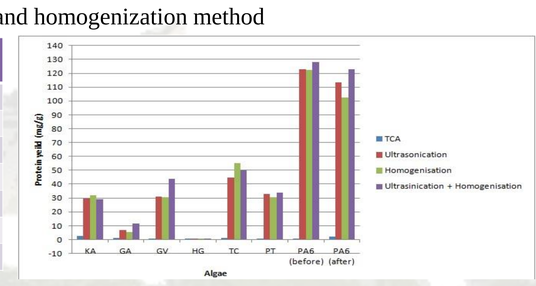
➢ Gas chromatography mass spectra of PUFAs extracted from microalgae and few macroalgae



GC spectra of P6 | GC spectra of KA | GC spectra of HG

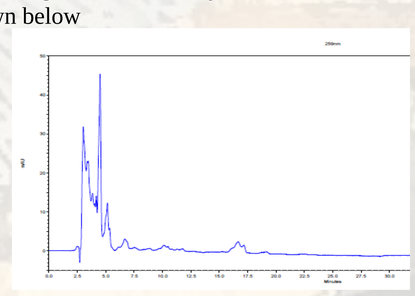
➢ Determination of protein by Kjeldahl, TCA, ultra sonication and homogenization method

Sample	TCA (mg/g)	Ultrasonication (mg/g)	Homogenisation (mg/g)	Kjeldahl method (%)
Kappaphycus alvarezii	2.7	29.6	31.8	46.9
Gelidiella acerosa	1.1	6.8	5.5	65.1
Gracilaria verrucosa	0.6	31.0	30.4	61.8
Halimeda gracilis	0.1	0.7	0.09	23.7
Turbanari conoides	1.1	44.6	55.3	52.1
Padina tetrastromatica	0.8	32.9	30.6	71.5
P6	0.4	122.9	122.3	187.4

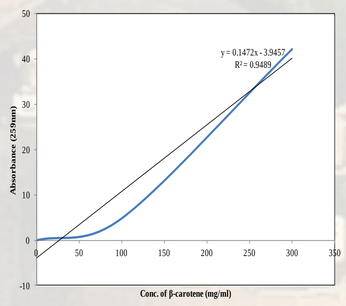


➢Total amount of carotenoid in microalgae sample was found to be 36.8% and concentration of β-carotene was found to be 10%.

➢ Carotenoids are extracted from microalgae consortia using Supercritical CO_2. Considering the complexity of carotenoid profile in microalgae, a gradient solvent system as described in method section, was developed to resolve a range of carotenoids as shown below



HPLC chromatogram of carotenoids extracted from microalgae using supercritical carbon dioxide



Standard curve for β-carotene

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