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AMINO ACIDS, FATTY ACIDS AND MINERALS IN *Kappaphycus* sps.

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ABSTRACT

Amino acid, fatty acid and minerals of *Kappaphycus sp*. were determined. Amino acids were estimated by HPLC method where as fatty acids by gas chromatography. Total 18 amino acids were found in the dried species, lysine is the major constituent and followed by asparagines, histidine, isoleucine, phenylalamine, tryptophan. In the case of fatty acids, eight components were identified including two components, namely, palmitic and cervonic acids are in traces. Alpha linolenic (n-3) and linoleic acids are found to be the major components. Macrominerals were identified by using flame atomic absorption spectrophotometry and found that red algae contained various macrominerals, namely, Sodium (23.4 mg), Potasium (12.44 mg), Magnesium (23.56 mg), Phosphorous (19.5 mg) per 1000mg and rich in Calcium (3.565 gm/100 gm). It was observed that Na/K ratio is below 2.0 which is interesting to note that *Kappaphycus sp*. could be used as a food supplement.

Keywords: seaweeds, red algae, *kappaphycus*, amino acids, fatty acids, minerals.

INTRODUCTION

Seaweeds have been widely used for human consumption in many parts of the world. Marine algae can serve as a source of minerals, vitamins, free aminoacids and polyunsaturated fatty acids. Macroalgae can be classified as red algae (Rhodophyta), brownalgae (Phaeophyta) or green algae (Chlorophyta) depending on their nutrient and chemical composition. Red and brown algae are mainly used as human food sources. Seaweed species are rich in beneficial nutrients, in countries such as China, Japan and Korea; they have been commonly utilized in human alimentation. Seaweeds have been consumed in Asia since ancient times. Further, marine algae have been utilized in Japan as raw materials in the manufacture of many seaweed food products, such as jam, cheese, wine, tea, soup and noodles and in the western countries, mainly as a source of polysaccharides for food and pharmaceutical uses [1, 2]. In Europe, there is an increasing interest in marine seaweeds as a food, nevertheless, at present there are no European Union specific regulations concerning their utilization for human consumption. Mineral content is generally high (8-40%) and the essential minerals and trace elements needed for human nutrition are present in seaweeds [2-4]. This wide range in mineral content, not found in edible land plants, is related to factors such as seaweed phyllum, geographical origin and seasonal, environmental and physiological variations [2]. Mineral content also depends on the type of seaweed processing and on the mineralization methods used. Ruperez [5] determined various mineral contents in several brown and red edible marine sea vegetables. Ke Li *et al*., [6] determined various chemical constituents of the red alga *Grateloupia turuturu*.

Seaweed mineral content is higher than that of land and animal products. In most land vegetables, ash content ranges from 5 to 10g/100g dry wt [7]. Sweet corn has a low content (2.6%) while spinach has a exceptionally high mineral content (20.4%) for a land plant. Thus edible seaweeds may be an important source of minerals, since some of these trace elements are lacking or very minor in land vegetables. Studies of fatty acids in seaweeds have investigated their seasonal variation differences among different plant tissues [8].

Protein aminoacids are usually considered to possess little, if any, taxonomic interest. Indeed, on account of their universal occurrence in living matter, variations of profiles are necessarily quantitative and plausibly affected not only by genetically controlled factors, but also by differences in physiological and environmental conditions. Consequently, most of the taxonomically oriented work has been devoted to the non-protein counterparts, supposedly of greater taxonomic potential. Nevertheless, evidence is being slowly accumulated that amino-acid profiles of protein extracts from different species show meaningful correlations. The distribution of unbound protein aminoacids has been given still less attention, although early investigation had provided some evidence of possible taxonomic value. Christine Dawczynski *et al*., [9] analysed the nutritional compositions of 34 edible seaweed products of various species such as *laminaria, undaria pinnatifida, hizikia fusiforme, porphyra*.

The determination of lipid composition in a given species is essential for further studies on lipid metabolism and on the effect of environmental factors. Moreover, some evidence suggests that the fattyacid and sterol composition may be useful for taxonomic purposes [10]. Rhodophyta are characterized by a high content of C_{20} polyunsaturated fatty acids, mainly arachidonic and eicosapentaenoic acids. Other abundant fatty acids in this class are palmitic and oleic acids. The major sterol of red algae, with a few exceptions, belongs to the type C_{27}. Cholesterol is the most commonly found and is usually present in the highest concentration. Two other C_{27} sterols

frequently detected in red algae are desmosterol and 22-dehydrocholesterol. The occurrence of C_{26}-, C_{28}, and C_{29}-sterols in varying quantities has been reported in a number of species of red algae [11].

The fatty acids are essential for nutrition of many animals, including humans and are of interest in biotechnology, food chain studies and cosmetics. Fatty acids of marine macrophytes have also attracted the attention of chemotaxonomists. Fatty acid compositions of red, brown and green macrophytic algae, belonging to different taxonomic classes, orders of families and genera, have distinguishing features of taxonomic value. Lipid profiles assist the assignment of algal taxonomic position, and also provide signature lipid profiles for use in organic geochemistry and food web studies. Such biochemical analyses may also prove useful in studying the abundance and ecology of these species in marine environments. From chemotaxonomic point of view lipids e.g. fatty acids and sterols provide interesting information for taxonomic purposes. Whereas higher plants have received extensive attention regarding lipid content, benthic algae have received relatively little attention and our knowledge is still rather poor. Some papers, however, have been published on the Rhodophyta lipid area, but the classification was confused, as in some cases especially, large variations occurred in the fatty acid or in the sterol composition within the same genera or sometimes within the same species.

About 30 species of red algae have been investigated for their fatty acid composition with controversial results. A majority of authors found that major fatty acids of Rhodophyta are the polyunsaturated 20:4w6 and 20:5w3, usually the latter is much more important, along with palmitic, myristic and oleic acids. Red algae have high concentration of eicosapentaenoic acid (C20:5, n-3, EPA) with 48.0-51.o% of total fatty acid methyl esters (FAME) and marginal concentrations of arachidonic acidwith 2.1%-10.9% of total fame and linoleic acidwotj 1.3-2.5% of total fame. In contrast, brown algae e.g.laminaria sp., have high concentrations of oleic acid with 4.1-20.9% of total fame, la with 4.0-7.3%0f total fame as well as alpha linolenic acid with 3.6-13.8% of total fame but low concentrations of epa with 5.9-13,6% of fame. Interestingly, in porphyrasp the concentrations of docosahexaenoic acid and docosapentaenoic acid were below the detection limit less than 0.1% of total fame.

In general, from the critical review of literature, it has been observed that the most studies on the nutrient contents of seaweeds have concerned fresh plants. Little is known of the effects of processing by drying or canning. The present investigation aims at on the following from *Kappaphycus sp*:

- Estimation of fatty acid profiles
- Determination of amino acid contents
- Evaluation of various minerals

MATERIALS AND METHODS

Samples were collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Algae samples were cleaned at epiphytes and necrotic parts were removed. Samples were rinsed with sterile water to remove any associated debris. Sample was kept under sunshade for 7 days. After drying the sample, it was ground thoroughly to powder form. The powder was then used for the primary estimation of fatty acids, amino acids and minerals. This powder was stored in cold conditions in an airtight container and analysis was carried out within three months of processing.

Qualitative and quantitative estimation of amino acids

Free and protein amino acids were estimated by O-pthaldialdehyde method described by Rajendra [12]. Extractions of free amino acids and soluble proteins from the algal tissues are described elsewhere. Concentrated 80% ethanolic extract was directly used for qualitative and quantitative estimation of free amino acids. For protein amino acids, protein in the extract was precipitated by adding equal volume of 10% TCA and dried in *vacuo*. To known quantities of dried protein (usually 75 mg), 2.0 ml of 6.0 N HCl was added and hydrolyzed at 110^{o}C for 18 hrs. After the hydrolysis, the hydrolysates were allowed to evaporate to dryness and the dried material was used for HPLC analysis.

Reagents

Borate buffer (0.4M)
Boric acid (2.47g) was dissolved in 100 ml of water and pH adjusted to 9.5 with 4.0N NaOH.

Methanol tetrahydrofuron
The reagent was prepared by the addition of 30 ml of tetrahydrofuron to 970 ml of methanol.

Ortho-pthaldialdehyde reagent
Anhydrous ortho-pthaldialdehyde (50 mg) was dissolved in a mixture of 2.0 ml of methanol, 8.0 ml of borate buffer and 5.0 ml of β-mercaptoethanol. The reagent was prepared fresh for every estimation.

Procedure

One milliliter of ortho-pthaldialdehyde reagent was added to 200 ml of the amino acid sample, mixed thoroughly and kept undisturbed for 2 minutes for derivatization. The sample was then filtered and 20 ml was injected in to the HPLC for analysis.

Operational condition of HPLC

The instrument: LACHROM L-700 and D-70000 HPLC
Column : C 18' 4.6 X 250 mm, 5 µm packing
Mobile phase A: 0.1 M acetate buffer (pH 7.2)
Mobile phase B: 3% tetrahydrofuron in methanol
Flow rate: 1.5 ml/min.
Gradient: 10 - 42% b for 15 min.,
42% B for 10 min.
42% - 50% B for 3 min.,

50% - 70% B for 7 min.
70% - 90% B for 4 min.,
90% - 100 % B for 1 min.
100% B for 2 min.
100% - 10% B for 1 min.
10% B for 2 min.

Detector : fluorescence, 9 µl flow cell F1-2
Excitation filter : 305-395 nm
Emission filter : 430-470 nm
Sensitivity : 0.005 Abs

AMINO ACIDS - METHOD OF ANALYSIS
Column : DENALI C18 5MICROMM
4.6 mm x 150 mm (cat no. DEN-5C18-15046)
Flow : 1ml/minute
Mobile Phase: A = 20 : 80 Acetonitrile : 25 Mm Potassium Phosphate, pH 3.3 B = 80: 20 CAN : 25 Mm Potassium Phosphate , pH 3.3
Gradient : 0 to 75% B over 15 min
Temperature : Ambient 23° C
Detection: 254 nm
Sample: 5 microlitre of aminoacids standards mixture

Estimation of fatty acids

Fatty acids in the samples were identified and quantified as methyl esters in NEON 11 gas chromatography instrument following the procedure outlined by Miller and Berger [13].

Sample preparation for lipids

One gram of shade dried powdered species was extracted with chloroform-methanol mixture (2:1 v/v) using a glass pestle and mortar and a pinch of glass powder. The homogenate was taken in a container, sealed air tight and incubated overnight in an over at 50°C. The sample was once again extracted as described above and filtered. The filtrate was added enough quantity of anhydrous sodium sulphate to dehydrate. The resulting concentrate was then taken in a Pasteur pipette, dried at room temperature by passing nitrogen gas and stored in a sealed airtight container. Fatty acids in lipid sample thus prepared were then converted to methyl esters in the following manner.

Reagents for preparation of fatty acid methyl esters (FAMES)

a) Saponification reagent: 45 g of NaOH in 300 ml of methanol:water mixture (1:1 v/v).
b) Methylation reagent: 325 ml of 6.0 N HCl mixed with 275 ml of CH_3OH
c) Extraction solvent: 200 ml of hexane mixed with 200 ml of anhydrous diethyl ether.
d) Base wash: 10.8 g of NaOH dissolved in 900 ml of distilled water.

Hundred milligrams of each sample was taken in Telfon-lined screw cap tube and 1.0 ml of reagent A was added. The tube was closed and boiled for 30 minutes with intermittent shaking. Two milliliters of reagent B was then added and boiled again in a water bath at 80°C for 20 minutes. Following for 30 minutes with intermittent shaking. Two milliliters of reagent B was then added and boiled again in a water bath at 80°C for 20 minutes. Following cooling of the tubes to room temperature, 1.25 ml of reagent C was added and the tube was rotated end to end for 10 minutes. After discarding the lower aqueous phase, 3 ml of reagent D was added and the tube was shaken thoroughly for 10 minutes. The upper layer, an organic phase containing the FAMEs was used for analysis by GC. Two microliters of each sample was injected in to the GC column and the fatty acids were identified and quantified by comparing the peaks with that given by standard fatty acid methyl esters. The column conditions are described below:

Instrument	:	NEON 11 GC
Column	:	DEGS 10%
Oven temperature	:	180°C isothermal
Injection port temperature	:	200°C
Detector	:	FID
Detector temperature	:	210°C
Carried gas	:	Nitrogen
Flow rate	:	30 ml/min

Identification of minerals

Estimation of various minerals such as copper, zinc, sodium, potassium, magnesium etc has been carried out as per the procedure outlined in standard manual. The detailed procedure for estimation of copper and zinc is given below. Similar procedure has been followed for estimation of other minerals except changing the corresponding standard solution.

Assay for copper

Copper standard stock solution- Dissolve about 1.00 g of copper foil in a minimum volume of a 50% (v/v) solution of nitric acid, and dilute with a 1% (v/v) solution of nitric acid to 1000mL. This solution contains 1000 µg of copper per mL.

Standard preparations - Transfer 10.0mL of Copper standard stock solution to a 100-mL volumetric flask, and dilute quantitatively with 0.125 N hydrochloric acid to volume to obtain a standard solution having a concentration of 100 µg of copper per mL. To separate 200-mL volumetric flasks, transfer 1.0, 2.0, 4.0, 6.0, and 8.0mL of the standard solution, dilute with water to volume, and mix to obtain solutions having known concentrations of about 0.5, 1.0, 2.0, 3.0, and 4.0 µg of copper per mL.

Assay preparation-Take serum equivalent to 2 µg of copper per mL

Procedure: Concomitantly determine the absorbances of the standard preparations and the Assay preparation at the copper emission line at 324.7 nm with an atomic absorption spectrophotometer equipped with a copper hollow-cathode lamp and an air-acetylene flame, using 0.125 N hydrochloric acid as the blank. Plot the absorbances of the Standard preparations versus concentration, in µg per mL, of copper, and draw the

straight line best fitting the five plotted points. From the graph so obtained, determine the concentration, C, in µg per mL of copper in the assay preparation. Calculate the quantity, in mg, of copper (Cu) in the fine powder of *kappaphycus sp*. by using the formula: 0.001CD, in which D is the dilution factor used to prepare the Assay preparation.

Estimation of zinc: by atomic absorption spectrometry

Zinc standard stock solution: Transfer about 311 mg of zinc oxide, accurately weighed, to a 250-mL volumetric flask, and add 80mL of 5 M hydrochloric acid, warming if necessary to dissolve. Cool, dilute with water to volume, and mix to obtain a solution having a known concentration of about 1000 µg of zinc per mL.

Standard preparations

Dilute a volume of the Zinc standard stock solution quantitatively and stepwise if necessary, with 0.125 N hydrochloric acid to obtain a standard solution having a known concentration of about 50 µg of zinc per mL. Transfer 5.0mL of this solution to separate 100-mL volumetric flasks. Dilute the contents of each flask with 0.125 N hydrochloric acid to volume, and mix to obtain solutions having known concentrations of about 2.5 µg of zinc per mL.

Assay preparation-Weigh sample 1 gram to 50 ml in 0.125 N hydrochloric acid.

Procedure

Concomitantly determine the absorbances of the standard preparations and the assay preparation at the zinc emission line at 213.8 nm with an atomic absorption spectrophotometer equipped with a zinc hollow-cathode lamp and an air-acetylene flame, using 0.125 N hydrochloric acid as the blank. Plot the absorbances of the Standard preparations versus concentration, in µg per mL, of zinc, and draw the calculate the quantity, in mg, of zinc (Zn) in the portion of sample.

Flame photometry

The following methods are based on the measurement of intensity of spectral lines emitted by elements such as sodium, potassium, calcium, etc. The substance containing the element is dissolved in an appropriate solvent (usually water) and subjected to excitation in a flame of appropriate temperature and composition.

Methods

Unless otherwise directed in the individual monograph, one or the other of the following methods may be used. In Method A, measurements are made by comparison of sample solutions with solutions containing a known amount of the element being analysed. Method B is suitable for samples which contain very small quantities of the element to be analysed or where there is interference from other elements.

Method A

Prepare a series of standard solutions containing the element to be determined in increasing concentration within the concentration range recommended for the particular instrument used. Choose the appropriate filters or adjust the monochromator to select the wavelength prescribed in the monograph. Spray water into the flame and adjust the galvanometer reading to zero. Spray the most concentrated standard solution into the flame and adjust the sensitivity so that a full-scale deflection of the galvanometer is recorded. Again spray water into the flame and when the galvanometer reading is constant readjust it to zero. Spray each standard solution into the flame three times, recording the steady galvanometer readings obtained and washing the apparatus thoroughly with water after each spraying. Prepare a calibration curve by plotting the mean of each group of three readings against the concentration. Prepare the solution of the substance being examined as prescribed in the monograph and adjust the strength, if necessary, to bring it into the range of concentration recommended for the instrument used. Spray the solution into the flame three times, recording the galvanometer readings and washing the apparatus thoroughly with water after each spraying. Using the mean of the galvanometer readings, determine the concentration of the element being examined from the calibration curve. To confirm the concentration thus obtained, repeat the operations with a standard solution of the same concentration as that of the solution being examined.

Method B

Place in each of not fewer than three similar volumetric flasks equal volumes of the solution of the substance being examined prepared as prescribed in the monograph. Add to all but one of the flasks a measured amount of the prescribed standard solution to produce a series of solutions containing regularly increasing amounts of the element to be determined. Dilute the contents of each flask to the required volume with water. Prepare the flame photometer in the manner described under Method A, using water for the adjustment to zero and the solution with the largest amount of added element to adjust the sensitivity. Examine each solution three times and plot the mean of the readings against concentration on a graph whose origin or zero reading represents zero concentration of the added element. Extrapolate the straight line joining the points until it meets the extrapolated concentration axis at a point on the negative side. The distance between this point and the origin represents the concentration of the element in the solution being examined.

Sodium solution FP

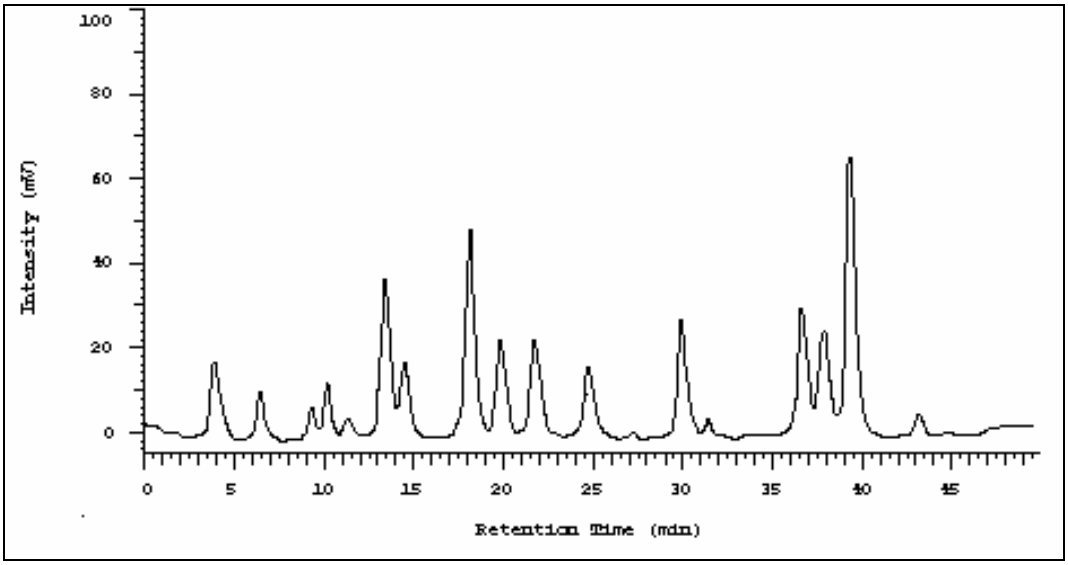
Dissolve 0.5084 g of sodium chloride, previously dried to constant weight at 130^{o} in sufficient freshly distilled water to produce 1000 ml.

Sodium Solution FP contains 200 µg of Na in 1 ml.

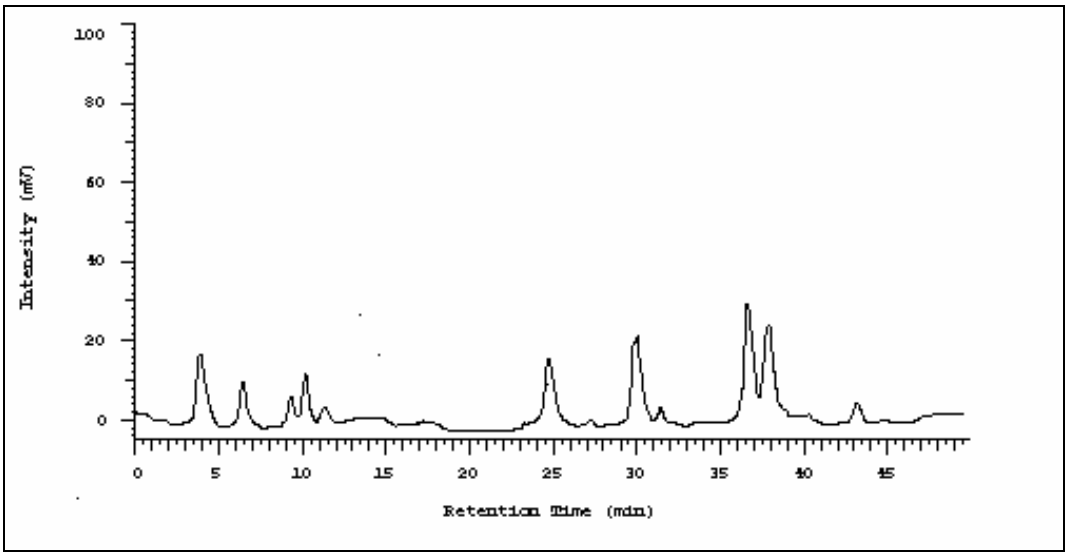
RESULTS AND DISCUSSIONS

Amino acid

The standard graph for Amino acid is shown in Figure-1. The various components available in the standard amino acid are given in Table-1. Figure-1 also shows the various components of amino acids available in *Kappaphycus* sp.



(a) Standard graph



(b) Kappaphycus sp.

Figure-1. Profile of amino acids.

Table-1. Components of standard amino acid and *Kappaphycus sp*.

Amino acid standard			**Amino acids in red algae** (Kappaphycus)		
Component name	**R.T.**	**Area** (%)	**R.T.**	**Area** (%)	**Various components /100gm**
Aspartic acid	4.03	6.343	4.05	1.490	0.565±0.01
Glutamic acid	6.47	2.546	6.48	0.657	1.12±0.012
Asparagine	9.23	1.365	9.24	0.434	1.045±0.021
Serine	10.22	3.067	10.21	0.897	0.1121±0.02
Gultamine	11.67	1.275	---	-----	------
Glycine	13.45	11.152	13.45	7.566	0.1012±0.015
Threonine	14.89	3.565	14.89	3.909	0.5067±0.01
Arginine	18.08	13.565	18.08	4.454	0.52012±0.02
Alanine	19.53	6.921	19.53	3.787	0.706±0.001
Cystine	21.67	6.787	21.67	0.399	0.2012±0.01
Tyrosine	24.76	4.556	24.76	4.375	0.3415±0.012
Histidine	27.25	0.245	27.25	0.699	1.15±0.02
Valine	29.90	8.343	29.90	3.787	0.314±0.001
Methionine	31.41	0.756	----	-----	----------
Iso-leucine	36.62	7.554	36.62	3.967	1.126±0.01
Phenyl alanine	37.93	4.620	37.93	9.898	1.454±0.02
Leucine	39.34	14.41	39.34	4.677	0.9067±0.002
Taurine	41.11	0.545	---	----	----------
Lysine	43.39	1.105	43.39	38.060	2.075±0.01
Proline	45.11	0.616	45.11	6.979	0.3235±0.012
Tryptophan	46.12	0.656	46.12	0.853	1.11±0.01
		100.000		100.000	

Table-1 also presents the quantity and qualitative information on the various components available in amino acid. From the Table-1, it can be observed that the standard deviation (SD) for all the components is less than 0.05.

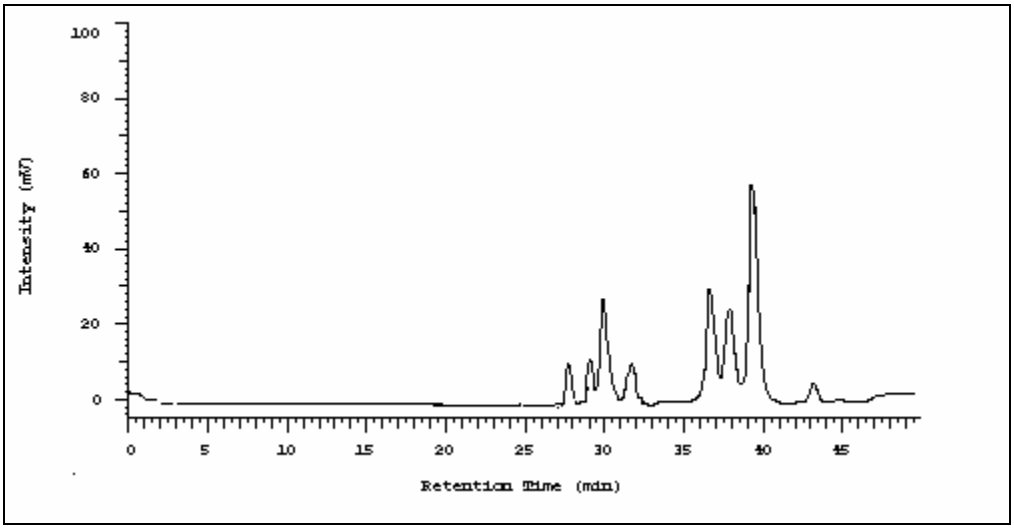
The dried sample of kappa.alvarezi was found to contain 18 amino acids, namely, asparagine, aspartic acid, glutamic acid, alanine, valine, glycine, arginine, serine, cystine, methonine, theronine, phenylalanine, tyrosine, isoleucine, leucine, histideine, lysine, tryptophan and praline. It can be observed from Table-1 that lysine content is the major component and the quantity being 2.075gms/100mgs. The other components, namely, phenylalanine (1.454gms), glutamic acids (1.12gms), isoleucine (1.126gms), histidine (1.15gms), tryptophan (1.11gms), methonine (1.078gm), asparagine (1.045gms) are followed by lysine. Further, it can be noted that glycine is much less (0.1012gms) quantity compared to all other components available in *Kappaphycus sp*.

Giuseppe Impellizzeri *et al*., [14] quantitatively determined free protein amino acids in 30 red algae. In most of the species, aspartic acid (asparagine), glutamic acid (glutamine), alanine, glycine and serine dominate, while massive accumulation of proline (up to 80.5%) was observed in six species, all belonging to the family of Rhodomelaceae. Giuseppe Impellizzeri [15] analysed 18 red algae sp for amino acids and low-MW carbohydrates using different methods. It was observed that all species of red algae examined had a similar composition of protein amino acids. All species contain low concentrations of basic amino acids and show a general prevalence of the same compounds (aspartic and glutamic acids, which often make up 50% or more of the total, alanine, glycine and serine). Christine Dawczynski *et al*., [9] examined different seaweed products for analysis of amino acids (aas), protein and dietary fibre. All essential aas were detected in the seaweed species tested and redalgae species featured uniquely high concentrations of taurine when compared to brown algae variteis. Dhamotharan [16] mentioned that **t**he dried samples of brown algae (*padina tetrastromatica and stocchosperumum marginatum*) were found to contain 18 aminoacids, namely, aspartic acids,

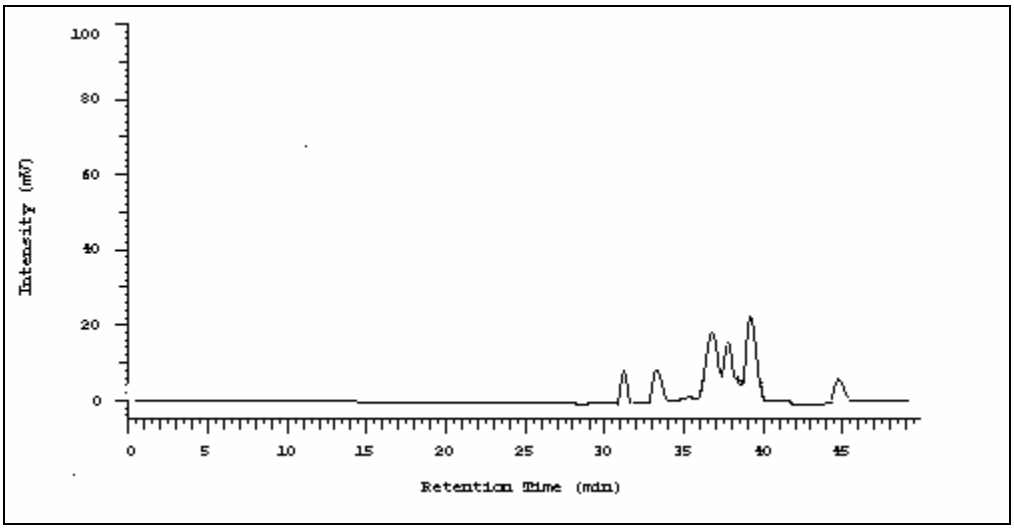
glutamic acid, asparagines, serine, glutamine, glycine, threonine, arginine, alanine, cystine, tyrosine, histidine, valine, methionine, isoleucine, phenyalanine, leucine and lysine). Analysis of amino acids by GC showed the presence of 18 amino acids in the tissues of the brown algae. In the present study showed that lysine is the major content of fatty acid. Interestingly, lysine was the major constituent in padina and accounted for 13% of the total amino acid content. Lysine is an essential aminoacid and has nutraceutical value. As compared to padina, the total amino acid content was 32% less with histidine as the major constituent in stoechospermum marginatum. Further lysine levels were relatively low in these tissues. The tissues of both algae showed high levels of asparagines in addition to having arginine and cystine indicating that these algae might be using asparagines as their transport form of N while arginine and cystine as the storage form. The aas fingerprint is distinct for the two algae. Dave and Chawhan [17] have reported high levels of lysine in the tissue of *caulerpa sp*. The observed levels of lysine in padina is more than that reported for the freshwater alga spirulina [18, 19] and less than that reported for another fresh water alga *dithophorooedogonia* [20]. Rajeshwar *et al*., [21] separated the ultraviolet (UV) absorbing mycosporine - like amino acids (MAAs) from a marine red alga *Gracilaria* cornea using HPLC. The isolated MAAs were identified as porphyra-334 and/or shinorine by comparing them with various standards. Their results indicated a highly stable nature of MAAs against the environmental stress factors like UV-B and heat. In general, the amino acid profile is important for evaluating the nutritional value of algae proteins, but the digestibility of algae protein was not analysed.

Fatty acids

The standard fatty acid plot is shown in Figure-2. Figure-2 also shows the profile of fatty acids available in *kappaphycus sp.* Timnodonic (EPA) and Cervonic (DHA) components were obtained through Figure-3. Table-2 shows the details of saturated fatty acids, mono unsaturated fatty acids and poly unsaturated fatty acids. From Table-2, it can be observed that there are eight fatty acid components are available in the *Kappaphycus sp*. The available saturated fatty acids include palmitic acid (in traces); stearic acid (0.1055 gm); mono unsaturated fatty acid is oleic acid (0.5065 gm) and poly unsaturated fatty acids are: linoleic acid (1.145 gm), α - linolenic acid (2.167gm), moroctic acid (identified), timnodonic (EPA) (0.1565gm) and cervonic (DHA) (in traces). In the analysed seaweed species, α - linolenic acid (C 18:3) was the most abundant saturated fatty acid (Table-2). Linoletic acid (C18:2) was the next abundantly available saturated fatty acid. Seaweed products represent an important source of long chain poly unsaturated fatty acid (LC-PUFA) (n-3, n-6) that are fundamental for the formation of important structural lipids and elements of cell membranes. In addition, these LC-PUFA are precursors of eicosanoids, which influence inflammation processes and immune reactions [22]. The two classes of PUFA have opposing physiological functions and their balance is important for normal growth and development. These FAs are beneficial for the prevention of cardiovascular diseases and other chronic diseases such as diabetes, hypertension and autoimmune diseases.



(a) Standrad



(b) *Kappaphycus sp*.

Figure-2. Profile of fatty acids.

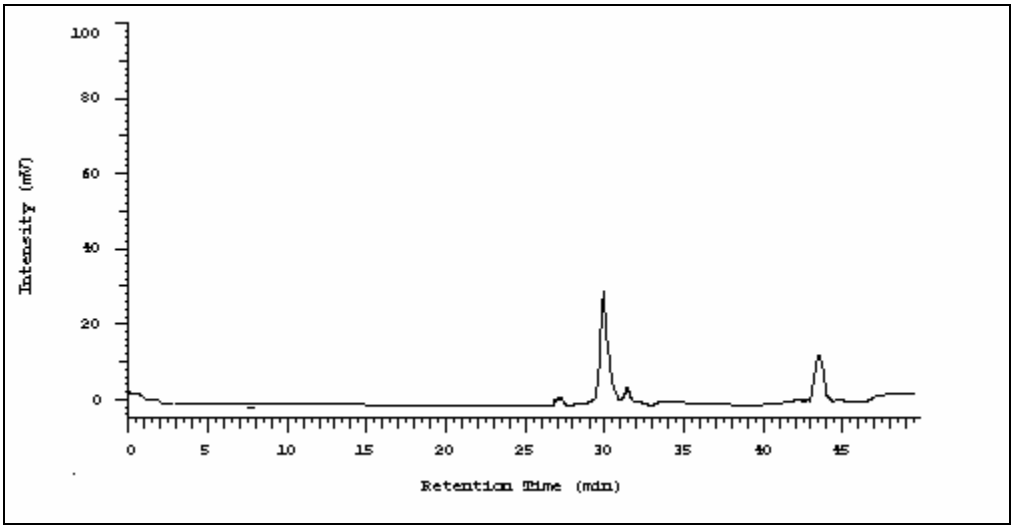


Figure-3. Profile of EPA and DHA components.

Table-2. Details of fatty acid components in standard and *Kappaphycus sp*.

No.	Fatty acid standard				Fatty acid in *Kappaphycus* sp.		
	Component name	**Carbon**	**RT**	**Area (%)**	**RT**	**Area (%)**	**Mean ± SD**
1	Myristic acid	C 14	26.8	6.95	-	-	---
2	Pentadecanoic	C 15	28.5	4.765	---	--	--
3	Palmitic acid	C 16	30.5	6.08	30.6	0.68	In traces
4	Moroctic acid	C 17	30.8	4.16			
5	Stearic acid	C 18	33.5	2.06	33.7	5.7	0.1055±0.02
6	Oleic acid	C 18 1	35.5	14.27	35.7	23.2	0.5065±0.012
7	Linoleic acid (n-6)	C 18 2	37.2	14.61	37.4	7.38	1.145±0.01
8	α - linolenic acid (n-3)	C 18 3	40.2	44.31	40.4	61.41	2.167±0.02
9	Moroctic acid	C 18: 4	44.5	0.84	46.2	1.63	identified
10	Burucic acid	C 22 1	45.4	0.238	---	---	---
11	Arachidonic acid	C 22 2	46.3	1.72	---	---	---
12	Timnodonic (EPA)	C 20.:5			30.89	60.35	0.1565±0.01
13	Cervonic (DHA)	C 20 : 6			43.5	39.65	In traces

The Europian nutritional societies have recommended that human diest with a n-6/n-3 ratio of 5:1 is health promoting (D-A-C-H, 2000). The result of this study show that the percentage of n-6 FA is comparatively lower compared to n-3 in *Kappaphycus sp.* The intake of food rich in n-3 LC-PUFA can have a positive influence on the composition of blood lipids and therefore be used for the prevention of arteriosclerosis. The fatty acid (FA) content varied strongly with in algal strains and therefore, a unique FA distribution for any given algal strain cannot easily be produced because lipid composition changes depending on various factors such as temperature characteristics, intensity of light, levels of minerals, nitrogen compounds and in the life cycle of the algae [23]. Xiancui Li *et al*., [24] examined the fatty acid compositions of 22 species of marine macrophytes by using capillary gaschromatography. It was observed that red algae had relatively high levels of the acids 16:0, 18:1 (n-7), 18:1 (n-9), 20:5 (n-3) and 20:4 (n-6) and those examined were rich in C_{20} PUFAS, these chiefly being arachidonic and eicosapentaenoic acids. The major fatty acids encountered in phaeophyta were 14:0, 16:0, 18:1 (n-9), 18:2 (n-6), 18:3 (n-3), 18:4 (n-3), 20:4 (n-6) and 20:5(n-3). C_{18} PUFAS are of greater abundance in the brown algae than in the red algae examined. In the present studies, it is found that C18:3 (n-3) is the major fatty acid of *Kappaphycus sp.* Peter *et al*., [25] reported the fatty acid and sterol composition of the controversial taxon *Heterosigma akashiwo* (two strains) as well as the typical raphidophyte *Chattonella antique*. It was found that the major fatty acids in both raphidophytes are 16:0, 18:4w3, 20:5w3, 16:1w7 and 14:0. Polyunsaturated fatty acids accounted for 46-50% of the total fatty acids in both species. Maurice Aknin *et al*., [26] investigated the composition of fatty acids of 12 algae species with capillary gas chromatography. More than 45 fatty acids were identified of which 16:0 20:5w3, 20:4w6, 16:1w7, 18; 1w9, 18; 1w7 and 14:0 predominate in most samples. The Solieriaceae was characterized by the presence of cyclopentyl and w-5 monounsaturated fatty acids. The large variations observed in the fatty acid as well as in the sterol compositions of red algae belonging to a same order or sometimes for a single may be explained by seasonal or geographical changes or eventually, by the presence in a given species of several varieties. Another explanation may be given by the effects of environmental factors. Christine Dawczynski *et al*., [9] analysed the nutritional compositions of 34 edible seaweed products of the *Laminaria sp., Undaria pinnatifida, Hizikia fusiforme* and P*orphyra sp.* Varities. Their study determined amino acid and fatty acid (FA) distributions and contents of protein, fat and total fibre of these seaweed varities. The marine macroalgae varieties tested demonstrated low lipid contents with 2.3±1.6g/100g semi-dry sample weight (s.w.) and proved to be a rich source of dietary fibre (46.2±8.0 g/100g s.w.). The protein content of seaweed products varied widely (26.6±6.3 g/100g s.w. in red algae varieties and 12.9±6.2 g/100g s.w. in brown algae varieties). All essential amino acids were detected in the seaweed species tested and red algae species featured uniquely high concentrations of taurine when compared to brown algae varieties. Fatty acid (FA) distribution of seaweed products showed high levels of n-3 FA and demonstrated a nutritionally ideal n-6/n-3 FA ratio. The predominate FA in various seaweed products was eicosapentaenoic acid which was at concentrations as high as 50% of total FA content. Similar observation was made in the analysis of fatty acid profile in *Kappaphycus sp.*

Tasende [27] isolated and identified fatty acids and sterols of gametophytes and sporophytes of *Chondrus crispus*. It was observed that the content of fatty acids and sterols in gametophytes (0.710 and 0.190 mg/g dry weight respectively) was higher than in sporophytes (0.622 and 0.113 mg/g dry weight). The main fatty acids were palmitic, palmitoleic, oleic, arachidonic and eicosapentaenoic acids. In the present study, it is found that Oleic acid is also one of the fatty acids of *Kappaphycus sp*. These five fatty acids represented more than 78% of all the fatty acid composition in both generations. In addition unsaturated fatty acids were present in a much greater quantity (>80%) than saturated fatty acids. However, differences in the distributions of fatty acids between the two generations were observed. Mohamed Fayaz, *et al*., [28] analysed *Kappaphycus alvarezzi*, an edible seaweed from the west coast of India for its chemical composition, iron bioavailability and antioxidant activity. Gas chromatographic analysis of sample extracts showed the presence of fatty acids mainly containing heptadecanoic acids, 17:0 (34.24%); *cis*-heptadeconic acids, 17:1 (13.50%); oleic acid, 18:1w9 (11.0%); linoleic acid, 18:2w6 (2.30%); linolenic acid, 18:3 (6.513%); and arachidonic acid, 20:4w6 (1.367%). *K. alvarezzi* has a higher composition of unsaturated fatty acids (44.5%) when compared to saturated fatty acids (37.0%). Similar observation was noted in the present investigation. Some of the seaweeds are known to contain odd number fatty acids [29].

Minerals

The following minerals are identified in *Kappaphycus sp*. (Table-3). It can be noted from Table-3 that Ca is the major constituent of algae and formed the bulk of total minerals.

Table-3. Various minerals identified in *Kappaphycus sp.*

Minerals (mg/kg)	
Sodium	23.4
Potassium	12.44
Calcium	3.565gm
Magnesium	23.56
Phosphorus	19.5
Iron	7.89
Copper	0.897
Zinc	1.464
Manganese	0.44
Molybdenum	0.677
Fluoride	0.056

The next major constituent is magnesium and sodium. Fluoride is the least mineral product of this algae. From the above, it can be observed that Na/K ratio is below 2.0 which is interesting from the point of view of nutrition, since the intake of sodium chloride and diet with a high Na/k ratio have been related to the incidence of hypertension. Further, it can be observed that the mineral content available in *Kappaphycus sp.* for human consumption is well within the limits (1.5-10 mg/100g; [1]). In general, algal product would supplement the daily intake of some trace elements for adults: Fe, 10-18 mg; Zn, 15 mg; Mn, 2.5-5 mg and Cu, 2-3 mg [1, 5] determined mineral content in several brown and red edible marine seed vegetables. Seaweeds contained high proportions of ash (21.1- 39.3 %) and sulphate (1.3 - 5.9%). In brown algae, ash content (30.1-39.3%) was higher than in red algae (20.6 - 21.1%). Atomic absorption spectrophotometry of the ashes indicated that marine seaweeds contained high amount of both macro minerals (8.083-17.875 mg/100g, Na, K, Ca, Mg) and trace elements (5.1 - 15.2 mg/100g, Fe, Zn, Cu, Mb), than those reported for edible land plants. In the present study, it was identified that Ca is the major mineral. Fayaz *et al*., [28] used the ash of the sample for the estimation of mineral elements (Calcium, iron, Zinc) by AOAC procedure. The concentrations of the elements in *K. alvarezzi* were determined with atomic absorption spectrophotometry. Triplicate determinations for each element were carried out. The concentration of the elements was determined from calibration. It was observed that K. alvarezzi contains calcium, 159.5, iron, 33.8 and zinc, 1.58 mg/100g of the sample. The presence of significant amounts of calcium and iron in K. alvarezzi may be due to its metabolic system in which it is capable of directly absorbing elements from the sea water. Dhamotharan [16] estimated various mineral content available in *stoechospernum marginatum and padina.* The total concentration of the minerals was found to be always high in padina (7244.0 µg/g dry wt.) as compared to that in stoecho (3091.0 µg/g dry wt). Ca was the major constituent of both algae and formed the bulk of total minerals. Similar observation was made in the present investigation. Ca levels in padina amounted to 87.0% of the total minerals while the same in stoecho was 66.0%. The next major constituent in these alge was iron. The iron content in *stoechospernum* was 27.0% of the total mineral content and in padina it amounted to 10.0%.

CONCLUSIONS

Amino acids, fatty acids and mineral contents were estimated on the *Kappaphycus sp*. Sample was collected from the sea coast of Rameshwaram, Tamil Nadu, India in the form of dry and living sample. Amino acids were estimated by using HPLC method where as fatty acids by gas chromatography. Total 18 amino acids were found in the dried powder of species. Among all the amino acids, lysine is the major constituent and followed by asparagines, histidine, isoleucine, phenylalamine, tryptophan. In the case of fatty acids, eight components were identified including two components, namely, palmitic acid and cervonic acid are in traces. Alpha linolenic acid (n-3) and linoleic acid are found to be the major components. The intake of food rich in n-3 long chain poly unsaturated fatty acid can have a positive influence on the composition of blood lipids and therefore be used for the prevention of arteriosclerosis. Macrominerals were identified by using flame atomic

absorption spectrophotometry and it was found that red algae contained various amounts of macrominerals such as Sodium (23.4 mg), Potasium (12.44 mg), Magnesium (23.56 mg), Phosphorous (19.5 mg) per 1000mg and rich in calcium (3.565 gm/100 gm). The studies showed that red seaweeds could be used as a food supplement to meet the recommended daily intake of some essential minerals.

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