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RESEARCH ARTICLE

ESTIMATION OF PROTEIN, CARBOHYDRATE AND MINERAL CONTENT IN SELECTED SEAWEEDS

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ABSTRACT

The present investigation is concentrated on Protein, carbohydrates and mineral content of *Spathoglossum marginatum, Padina tetrasmatica, Turbinaria conoides, Ulva fasciata and Enteromorpha flexuosa* for future applications in dietary supplements or food industries. Three seaweed samples (i) *S. marginatum* (ii) *P. tetrasmatica* (iii) *T. conoides* were collected from Mandapam coast in Rameshwaram and the two seaweeds (iv) *U. fasciata* (v) *E. flexuosa* were collected from Kovalam coast of Chennai, Tamilnadu, India. Of the above seaweeds tested *S. marginatum* showed maximum content of protein and carbohydrate. T. conoides showed maximum content of calcium, potassium and sodium, while *U. fasciata* showed maximum content of magnesium and iron. *P. tetrasmatica* observed maximum potassium content than other seaweeds tested

INTRODUCTION

Seaweeds are one of the commercially important marine living and renewable resources of India. They are known to contain more than 60 trace elements, minerals, protein, iodine, bromine, vitamins and several bioactive substances of economic value. Thus, they serve both as feeding and breeding grounds for invertebrates and fishes (Krishnamurthy, 2005). There are 20,000 species found distributed globally. Seaweeds are traditionally consumed in the orient as part of the daily diet. Currently, human consumption of green algae (5%), brown algae (66.5%) and red algae (33%) is high in Asia, mainly Japan, China and Korea (Karthikai Devi *et al*., 2009). However demand for seaweed as food has now extended to North America, South America and Europe. The different species consumed present a rich nutritional value as source of proteins, carbohydrates, minerals and vitamins. Seaweeds are potentially good sources of minerals, proteins, polysaccharides and fibre (Reeta Jayasankar *et al.,* 1990). Studies on the biochemical constituents such as protein, carbohydrate and lipid in green and brown marine algae have been carried out from different parts of Indian coast. Reports on certain edible seaweeds showed that many contain significant amount of protein, vitamins and minerals which are essential for human nutrition. The high vitamin and mineral contents of edible seaweeds make them nutritionally valuable.

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Most studies on nutritional evaluation were carried out from naturally collected seaweeds from many parts of the world (Sobha *et. al.,* 1992). The present investigation concentrated on Protein, carbohydrates and mineral content of *Spathoglossum marginatum*, *Padina tetrasmatica*, *Turbinaria conoides*, *Ulva fasciata* and *Enteromorpha flexuosa* for future applications in dietary supplements or food industries.

MATERIALS AND METHODS

Seaweed collection and processing

Three seaweed samples (i) *Spathoglossum marginatum* (ii) *Padina tetrasmatica* (iii) *Turbinaria conoides* were collected from Mandapam coast in Rameshwaram and the two seaweeds (iv) *Ulva fasciata* (v) *Enteromorpha flexuosa* were collected from Kovalam coast of Chennai, Tamilnadu, India. The algal sample was handpicked and washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes. It was kept in icebox containing slush ice, transported to the laboratory and washed thoroughly using tap water to remove the salt on the surface of the sample. The water was drained off and the algal material was spread on blotting paper to remove excess water. They were shade dried. The dried seaweeds were finally pulverized in the commercial grinder and the powdered seaweed samples were stored at 4°C and used for further analysis.

Extraction of sample

Each ground sample was weighed and transferred into a beaker. Distilled water was added in the ratio of 1:10 and stirred for 1 h with the aid of a magnetic stirrer. The extraction mixture was left to sediment for at least 1 h before the extract was separated from the residue by filtration through What man No. 1 filter paper. The residue was re-extracted twice, and the two extracts were combined. Extracts were produced in duplicates and used to estimate the protein, Carbohydrate and mineral content.

Protein estimation

Protein estimation was done as described by Lowry and Lopaz (1946), using Bovine serum albumin at the rate of 1mg/ml as the standard. The blue colour developed by the reduction of the phosphomolybdic phosphotungstic components the folin-ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartrate are measured in the Lowry's Method. Different concentrations of the standard ranging from 0.1 to 1 mg/ml were taken and made up to 1 mg/ml. Then 5ml of alkaline copper reagent was added, mixed well and allowed to stand for 10 min at room temperature. Then 0.5 ml of diluted Folins phenol reagent was added and mixed well. The mixture was incubated for 30 minutes at room temperature. The absorbance at 650 nm was read spectrophotometrically. The protein concentrations of seaweeds extracts were estimated

Carbohydrate estimation

Carbohydrates are hydrolyzed into simple sugars using dilute hydrochloric acid. In an acidic medium glucose is dehydrated to hydroxyl methyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm. Various concentration of the working standard solution in a serious of the test tube from 0.2 ml to 1 ml was prepared (10µg to 100 µg). Make up the volume 1 ml with distilled water. All the tubes are kept in an ice bath and slowly 5 ml of anthrone reagent was added and mixed properly. Green colour was developed and measured the optical density at 620nm.

Estimation of Sodium (Na), Potassium (K) and Calcium (Ca)

The estimation of Sodium (Na^+), potassium (K^+), and calcium (Ca^{+2}) were done by using a Flame photometer (Elico, India) by selecting suitable filter against deionised water as a blank. Sample of selected seaweed extracts were analysed for element detection using mixed standard and double distilled water as a reference. Here instrument is giving concentration of elements in millimole / liter. Calculate the amount of elements present in terms of percentage dry seaweed sample powder.

Estimation of Magnesium

The homogenized sample of selected seaweed powder (1.0gm) was placed in a 100-ml volumetric flask, and mineralized by addition of 5.0 ml of concentrated HNO_3 and heating at 90◦C for 45 min in a sand mineralization block. Another 5.0 ml of 4:1 mixture of HNO_3 and $HClO_4$ was added and heating continued at 130◦C for an additional 2 h until the sample was completely mineralized. Then the mixture was cooled and the resulting solution diluted to 25 ml with ultra pure water. A second dilution was prepared by taking different aliquots of the previous dissolution and diluting them with ultra pure water. Magnesium determination was carried out by direct aspiration into the flame atomic absorption spectrophotometer.

Estimation of Iron

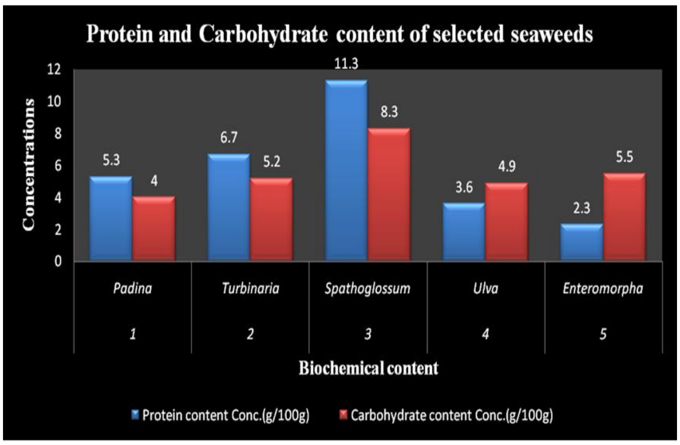
One gram of the dried sample of selected seaweed was individually weighed in a porcelain crucible and ignited at 600ºC in muffle furnace for 4 hours to get carbon free ash. The crucible containing the ash was allowed to cool at room temperature. Ten mL of hydrochloric acid (2N) was added to the ashes and mixed with a glass rod. Deionised water was used as a blank solution. Buffered chromogen (3mL) was added to 100 µL of ashes, blank solution and to 50, 100 and 200 µL of iron working standard solutions. The solution was allowed to stand for 20 minutes and the colour developed was read at 540 nm against a reagent blank in a Double beam UV visible spectrophotometer (Chemito, India). The amount of total iron content present in each of the sample was determined from the standard graph prepared with different concentrations of iron solution ranging from 0.5 to 2 □g/mL and the results were expressed in parts per million (ppm).

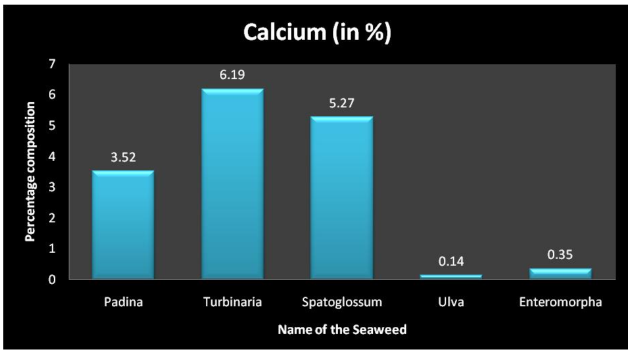
RESULTS

Among the five algae studied, results from Table 1 showed that both the biochemical constituents protein (28.29 mg. g^{-1}) and carbohydrate (8.3 mg. g^{-1}) are found to be maximum in *S. marginatum* whereas minimum protein content (2.3 mg. g^{-1}) and carbohydrate content (4.0 mg. g^{-1}) were recorded in *E. flexuosa* and in *P. tetrasmatica* respectively.

Table 1. Estimation of Protein and Carbohydrate content in the selected seaweeds

S.No	AlgaeSample Tested	Protein content Conc.(g/100g)	Carbohydrate content Conc.(g/100g)
1	*P. tetrasmatica*	5.3	4.0
2	*T. conoides*	6.7	5.2
3	*S. marginatum*	11.3	8.3
4	*U. fasciata*	3.6	4.9
5	*E. flexuosa*	2.3	5.5

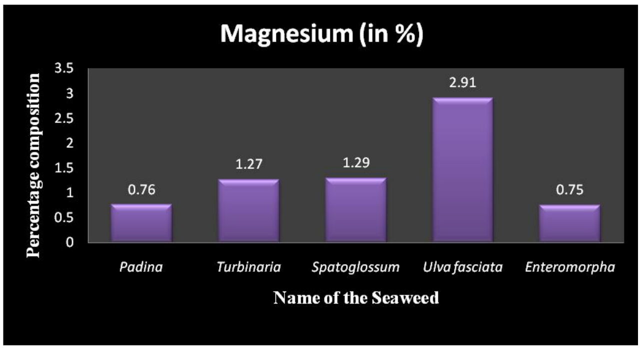




Determination of Calcium

Calcium was found maximum in *T.conoides* (6.19) and minimum in *U. fasciata* (0.14).

The variation in the calcium content of these seaweed species was recorded as *T.conoides* > *S. marginatum* > *P. tetrasmatica* > *E. flexuosa* > *U. fasciata* (Table 2).



Determination of Magnesium

Of the five seaweeds taken up for mineral analysis Magnesium content of *U. fasciata* (2.91) was found to be maximum. The difference in the mineral composition of Mg in the five seaweed species was observed as *U. fasciata* > *S. marginatum* > *T. conoides* >*P. tetrasmatica* > *E. flexuosa* (Table 2).

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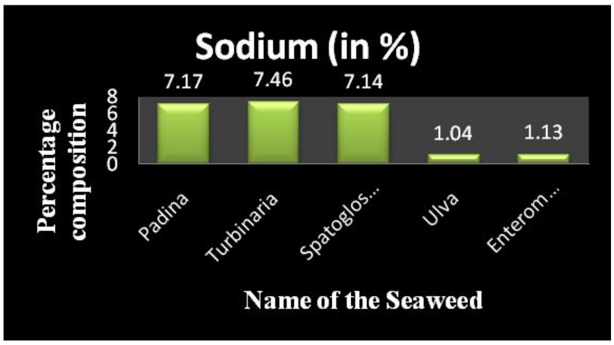
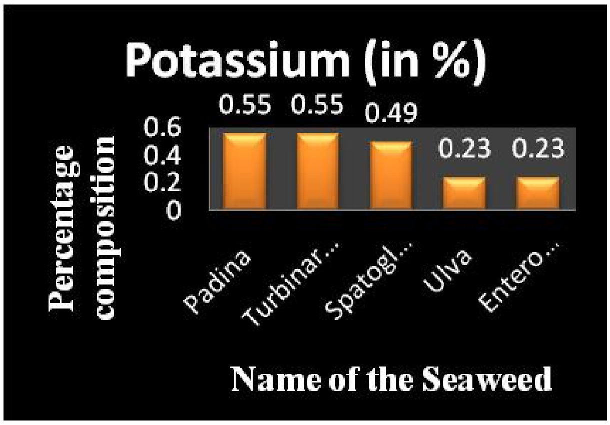
Determination of Iron

The nutrient content Iron was recorded to be maximum in *U. fasciata* (2.09) and minimum in *E. flexuosa* (0.07). The hierarchy of iron content in the five seaweeds studied is as follows: *U.fasciata* > *S. marginatum* = *T. conoides* > *P. tetrasmatica* > *E. flexuosa* (Table 2).



Determination of Sodium

Of the five seaweeds studied, one of the nutrient element sodium was found to be less in green seaweeds *U.fasciata* (1.04) and *E. flexuosa* (1.13) whereas in brown seaweeds *T. conoides* (7.46), *P.tetrasmatica* (7.17) and *S. marginatum* (7.14) sodium content was recorded high. Na content of the five seaweed varies as *U. fasciata* > *E. flexuosa* > *S. marginatum* > *P. tetrasmatica* > *T. conoides* (Table 2).



Determination of Potassium

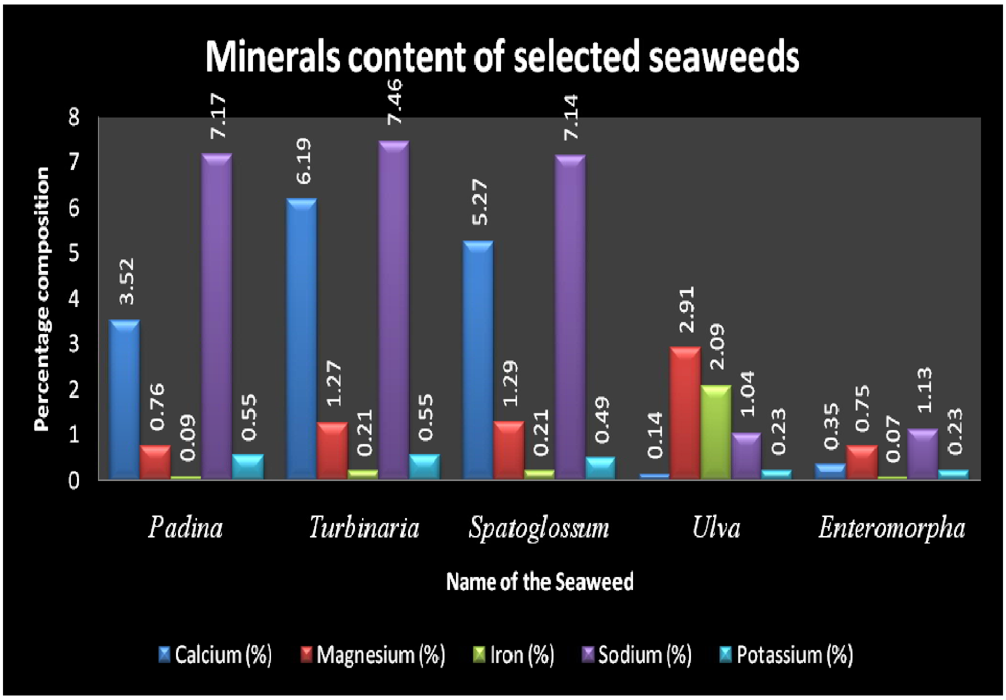
Maximum content of the nutrient potassium was registered in brown seaweeds *P. tetrasmatica* (0.55) and *T. conoides* (0.55), *S.marginatum* (0.49) than in green seaweeds *U. fasciata* (0.23) and *E. flexuosa* (0.23). The variations in the potassium content of the five seaweeds follows the pattern brown seaweeds *P. tetrasmatica* = *T. conoides conoides* > *S. marginatum* > green seaweeds *U. fasciata* = *E. flexuosa*.

DISCUSSION

Investigations on the sources of protein for fish, animal and human diet is the current trend of research. This study is an attempt on the analysis of biochemical and nutrient content of five different seaweeds such as *P. tetrasmatica, S. marginatum, T.conoides, U. fasciata and E. flexuosa.* Protein content of brown seaweeds was found to be higher than green seaweeds in the present research. similar results were observed in seaweeds by Ganesan and Kannan, 1994. This might be attributed due to the variations in the geographical locations of these brown and green seaweeds. Higher protein content might be due to higher temperature, lesser amount of protein content may be due to the use of protein by the seaweeds for their growth and reproduction.

Table. 2. Estimation of minerals content from selected seaweeds of *P. tetrasmatica, S. marginatum, T.conoides, U. fasciata and E. flexuosa*

Minerals	*P. tetrasmatica*	*T. conoides*	*S. marginatum*	*U.fasciata*	*E.flexuosa*
Calcium (%)	3.52	6.19	5.27	0.14	0.35
Magnesium (%)	0.76	1.27	1.29	2.91	0.75
Iron (%)	0.09	0.21	0.21	2.09	0.07
Sodium (%)	7.17	7.46	7.14	1.04	1.13
Potassium (%)	0.55	0.55	0.49	0.23	0.23



Comparatively carbohydrate content was found to be lesser than protein content in brown seaweed while higher in green seaweeds. The high amount of carbohydrate was probably due to the increased availability of nutrients utilized by the green seaweeds brought to the sea by land run off during rainy seasons. Among the various ecological parameters, salinity, dissolved oxygen, nutrient content and the amount of light penetration more effectively enhances the biochemical constituents of the seaweeds (Ganesan and Kannan, 1994). Seaweeds are well known for their ability to accumulate mineral ions and salts from seawater. They are placed in weeds in 'water free space' and 'donnan free space' (Eppley, 1962). Marine algae contain more than 60 trace elements in concentrations which are much higher than what is found in terrestrial plants (Bindu *et al., 2006*). In the present study maximum amount of sodium and potassium was found in *T. conoides, P. tetrasmatica* and *S. marginatum* than in *U. fasciata* and *E. flexuosa.* Bindu *et al., in* 2006 and Gangadevi (1997) reported similar accimilation pattern of seaweeds for sodium and potassium which can be attributed due to its negatively charged polysaccharides with high charge density, mainly alginate and various sulphated fucose containing polysaccharides (Haug, 1974). The seaweeds in the present analysis exhibited a preferential accumulation of potassium over sodium, which can be attributed due to the difference in the geochemical reactivities between two similar elements (Eppley, 1962).

It can also be due expressed that the geochemically more reactive potassium has a better chance to enter the biological system than the less reactive sodium. The potassium-sodium ratio was found to be higher than one for the algae which is partially in agreement with the studies of Gangadevi (1997). Na:K ratios are usually below 1:5 (Ruperez, 2002). Magnesium content was found to be minimum in *E. flexuosa, P. tetrasmatica* and *U. fasciata* when compared to the other two algae, and the calcium content was found to be moderate in all the algae, the intensity varies with the type of algae.

Minimum accumulation of Mg and Fe elements may be due to the climatic season which leads to the increase in osmoregulation activities, accumulation of Magnesium element in the seaweed might be minimum due to exceeding amount of silicon and Cl concentration in the ambient seawater. Content of Iron was found to be more in *U. fasciata* which was found to be comparable to the findings of Abirami and Kowsalya (2011). In seaweeds, Ca and Mg are the predominant elements of the nutrients uptake binding sites which enhance accumulation of si and cl by their stress in binding sites (Munda and Hudnik, 1991). Iron content was found to be comparitively high in *T.conoides* and *S. marginatum* when compared to *P. tetrasmatica, U. fasciata* and *E. flexuosa.* Accumulation of iron is supposed to be due to the leaching of iron from the vessels used for transport and fishery Gangadevi (1997). Sobha *et al.* (1988) showed that high value of iron content in U.fasciata, this might be due to the variations in seasons and geographical locations.

Walland (1981) and Wahbeh (1997) stated that the biochemical and nutrient contents of the seaweeds varies with factors such as season, habitat, age, plant part and growing conditions especially temperature, light, nutrients and salinity.

Conclusion

Seaweed consumption may be useful in the case of expectant mothers, adolescents and elderly persons those are all exposed to a high risk of calcium deficiency. High magnesium levels present in seaweed act as a natural relaxant and anti-inflammatory. Lignins are found in seaweed and act as a weak oestrogen and along with magnesium may help to restore normal sleep patterns and reduce hot flushes.

Rama Rao in 1992, Myklestad 1964, Smart *et al.,* 1992 proved that seaweeds are not only used as a fertilizer but very good soil conditioners, alters the nature of the soil and soil texture, improves the water holding capacity, stimulates the capillary action of the root system, mobilize trace elements in soil, decomposition of seaweeds has beneficial effects on the modification of soil micro flora and fauna.

Large number of nitrogen fixing organisms heaps from rotting seaweeds, humic acid and alginate improves the crumb structure and moisture-retaining and water holding characteristic of soil and ameliorate the sticky nature of clay soil. Seaweeds form unconventional resources of protein for fish, animal and human diet. Our studies show that green seaweeds increase the carbohydrate and magnesium content while brown seaweeds are rich in protein, potassium and sodium levels. They can be reduce the deficiency in higher tropic organisms.

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