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Elemental composition of selected edible seaweeds using SEM- energy dispersive spectroscopic analysis

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Abstract

Seaweeds are one of the important marine living resources in the world. Seaweeds are valuable food resource which contains high amount of minerals, vitamins, essential amino acids and dietary fiber. In the present study six seaweeds namely *Ulva lactuca, Ulva reticulata, Stoechospermum marginatum, Acanthophora spicifera, Gracilaria corticata* and *Gracilaria edulis* were selected for investigation of the chemical elements present in the cell wall using EDAX measurement. Mineral composition such as calcium, sodium, potassium and iron were analyzed in the selected seaweeds. The study reveals that Ulva lactuca contains the following order of elements C> O> Mg> S> Si>Ca> Na> Al> K>Cl> Se and in *Ulva reticulata* contains O> C> S> K>Cl> Na> Mg>Ca> Si> Al> Fe. Whereas *Stoechospermum marginatum* showed the presence of elements in the following order Cl> C> O> Na>Ca> S>Si> Al and *Acanthophora spicifera* showed the eight elements in the following order O> C> K>Cl> S>Ca> Na> Mg. *Gracilaria corticata* contain C> O>Cl> K> S> Na> Si> Fe> Al> Mg and *Gracilaria edulis* contains O> C> K>Cl> S>Ca> Na> Si> Mg> Al respectively. Quantitative analysis of the mineral composition of the selected seaweeds was as follows in *Gracilaria edulis* calcium was (3603.2±362.2) and potassium was (6021±30.8). *Ulva lactuca* had the maximum amount of sodium (2291±12.91). Iron was detected in *Gracilaria corticata* and was found to be 64.4±3.7 mg. From the quantitative analysis of the mineral composition of the selected seaweeds were different from that of the measurement. The different may be attributed due to the variation in sample preparation. Thus it is concluded that the EDAX helps to measure the elements presents on the superficial layer and provides an overview of the probable elements that are presents. The results of the present study reveals that the elements like calcium, sodium, potassium and magnesium were commonly present in the selected seaweeds. Seaweeds will surely find an important place in the food industries as a nutrient fortifier to combat the micronutrient deficiencies among the population.

Introduction

Minerals are inorganic substances present in all body tissues and fluids and their presence is necessary for the maintenance of certain physiochemical processes which are essential to life (Soetan *et al*., 2010). Inorganic substances like S, Ca, Mg, Si, Na, K are essential for daily functions and defense system. Minerals cure body aliments and make energy for bone strength and cell organelle functions. Trace elements balance the living system for biochemical and nutritional aspects (Balakrishan *et al*., 2013). Seaweeds are multicellular macro algae used as potential renewable resource in the field of medical and commercial environment. Seaweeds are commonly categorized in to three groups such as Chlorophyceae (Green seaweeds) Phaephyceae (Brown seaweeds) and Rhodophyceae (Red seaweeds) especially on the basis of the pigments and the stored food materials (Jagadeesan *et al*., 2010). The seaweed contains numerous pharmacologically important bioactive constituents such as flavonoids, carotenoids, dietary fibre, protein, essential fatty acids, vitamins and minerals. Many of the compounds are used to treat disease like cancer, Acquired Immune Deficiency Syndrome (AIDS), inflammation, pain, arthritis as well as viral, bacterial and fungal infections. Nowadays seaweeds are used as dietary food supplements in daily life to regulate the human health (Ganesan *et al*., 2007; Cynthia *et al*., 2011). Seaweed contains high ash content indicating appreciable amounts of minerals. Mineral content of seaweed can account for up to 36% of its dry mass and the macro minerals include sodium, calcium, magnesium, potassium, chloride, sulfur and phosphorus whereas the micro minerals include iodine, iron, zinc, copper,

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Table 1. Elemental composition of seaweeds using SEM-EDAX during Mon soon present in the cell wall of the selected seaweeds

Elements	Weight of element (%)					
	Ulva lactuca	*Ulva reticulata*	*Stoechospermum marginatum*	*Acanthophora spicifera*	*Gracilaria Corticata*	*Gracilaria edulis*
C	47.49	17.66	19.61	20.62	79.90	24.96
O	43.90	42.94	9.37	30.97	14.71	29.43
Mg	2.66	3.86	-	1.17	0.08	0.84
S	2.54	10.14	1.14	7.92	0.64	7.82
Si	1.45	2.90	0.84	-	0.26	1.48
Na	0.69	4.88	4.46	4.98	0.48	3.81
Ca	0.79	3.00	2.11	5.82	-	5.42
Al	0.32	1.78	0.38	-	0.11	0.53
K	0.10	6.48	-	16.62	1.68	17.35
Cl	0.06	4.90	62.02	12.26	1.89	8.36
Se	0.01	-	-	-	-	0.00
Fe	-	1.47	-	-	0.25	-
Total	100.00	100.00	100.00	100.00	100.00	100.00

selenium, molybdenum, fluoride, manganese, boron, nickel and cobalt (Niranjan and Se-Kwon Kim, 2011). Marine algae contain more than 60 trace elements in a concentration much higher than in terrestrial plants (Kannan, 2014).

Seaweed could be used as a food supplement in order to reach the recommended daily intakes of some macro minerals and trace elements (Rúperez, 2002). The present research work deals with detection of the elemental composition of six species of seaweeds including Chlorophycae (*Ulva lactuca* and *Ulva reticulata*) and Phaephyceae (Stoechospermum marginatum) and Rodophyceae (*Acanthophora spicifera, Gracilaria carticata* and *Gracilaria edulis*) using SEM - Energy Dispersive Spectroscopic and quantitative analysis of the selected elements using standard procedure.

Materials and Methods

Collection of seaweeds

Samples of the six seaweeds *Ulva lactuca, Ulva reticulata, Stoechospermum marginatum, Acanthophora spicifera, Gracilaria corticata* and *Gracilaria edulis* were collected from Pamban coastal area Rameswaram in south India in month of October to January. The seaweeds were thoroughly cleaned with seawater and finally cleaned with running water to remove salt and foreign materials. The cleaned seaweeds were dried and ground into fine powder and it was stored at room temperature and used for further analysis.

Preparation of SEM studies in seaweeds sample

X-Ray microanalysis was done with an EDAX (Energy Dispersive Spectroscopic Analysis) Bruker Nano German. EDAX identified the elements present in the cell wall of the six seaweeds. The weight of the elements and their atomic weight (%) were estimated. The energy dispersive X-ray microanalysis provides a unique approach for obtaining qualitative and quantitative compositional analysis of individual cell and intra cellular compartment to localize distribution of elements of leaf differed not only by quality but also in quantity (Sundari and Selvaraj, 2009).

Mineral composition

Mineral contents such as calcium, sodium, potassium were determined using flame photometer and iron was determined by spectrophotometer (Raghuramulu *et al.*, 2003) the determination was carried out in triplicate.

Results and Discussions

Element constituents in cell wall of the selected seaweeds

The results of energy dispersive X- ray

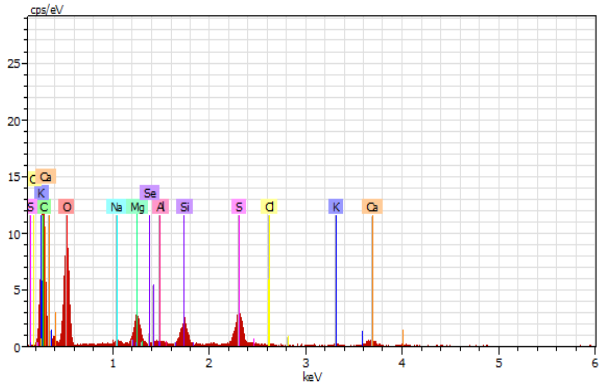

Figure 1. Energy Dispersive Spectroscopic analysis of *Ulva lactuca*

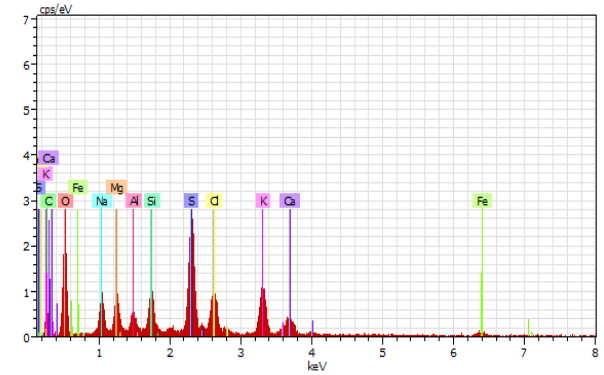

Figure 2. Energy Dispersive Spectroscopic analysis of *Ulvareticulata*

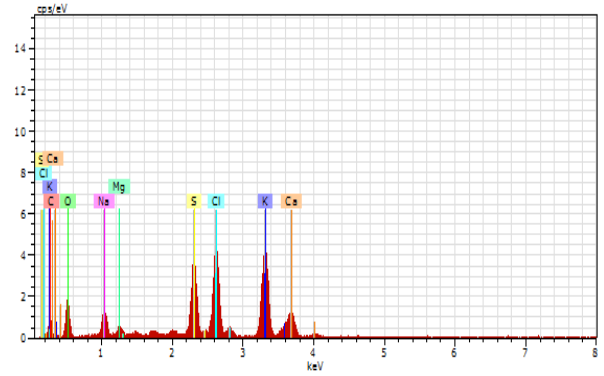

Figure 3. Energy Dispersive Spectroscopic analysis of *Acanthophoraspicifera*

spectrum analysis showed the presence of different chemical elements in the cell wall of *Ulva lactuca, Ulva reticulata, Stoechospermum marginatum, Acanthophora spicifera, Gracilaria corticata* and *Gracilaria edulis*. The order of the nine chemical elements from epidermal portion of the *Ulva lactuca* (Figure 1) namely C> O> Mg> S> Si>Ca> Na> Al> K>Cl> Se were observed respectively. The weights of different elements were given as percentage (%) values. Among the nine elements, the maximum contribution from carbon was 47.49% and the least amount 0.01% of selenium was recorded. Contributions of other elements like oxide 43.96%, magnesium 2.66%, sulphur 2.54%, silicon 1.45% sodium 0.69, calcium 0.79 and potassium 0.10 were respectively detected.

Ulva reticulata (Figure 2) O> C> S> K>Cl> Na> Mg>Ca> Si> Al> Fe were noticed. 4.88% of sodium, 6.44% of potassium, 3.86% of magnesium was detected. *Stoechospermum marginatum* showed the presence of elements in the following order Cl> C> O> Na>Ca> S>Si> Al with maximum amount of chlorine (62.07%) and the minimum amount of aluminum (0.38%) and other elements like copper (19.61%), oxide (9.37%), sodium (4.46%), calcium (2.11%), sulphur (1.14%) and silicon (0.84%) were detected.

Acanthophora spicifera (Figure 3) showed the eight elements in the following order O> C> K>Cl> S>Ca> Na> Mg and in *Gracilaria corticata* and *Gracilaria edulis* contain C> O>Cl> K> S> Na> Si> Fe> Al> Mg and O> C> K>Cl> S>Ca> Na> Si> Mg> Al respectively. Maximum amount of oxide was detected in *Acanthophora spicifera* (30.97%) and followed by *Gracilaria edulis* with (29.43%). Magnesium presents 1.17% in *Acanthophora spicifera*, 0.08% in Gracilaria corticata and 0.84% in *Gracilaria edulis*. Calcium was found to be 5.82% in *Acanthophora spicifera* and 5.42% in *Gracilaria edulis*. *Gracilaria corticata* had 0.25% of iron.

Mineral composition of selected edible seaweeds

The Table 2 shows that the macro mineral calcium, sodium, potassium and the micro mineral iron was quantified in this study and reported that among these elements calcium was detected in higher amount in *Gracilaria edulis* (3603.28±362.2) and *Ulva lactuca* (2256±2302). Whereas *Acanthophora spicifera, Stoechospermum marginatum, Ulva reticulata* and *Gracilaria corticata* were contains 479±14.46, 455.7±17.47, 361.6 ±17.3 and 339.61±12.91 mg of calcium respectively.

Sodium content of the selected seaweed ranged from 348±16.25 to 2291±12.91. The maximum amount of sodium was present in *Ulva reticulata* and the minimum amount was detected in *Ulva lactuca*. Potassium content of the selected seaweed was 152.7±8.35, 4259±61.15, 700±8.66, 2633±30.5, 871.3±30 and 6021±0.8 in *Ulva reticulata, Ulva lactuca, Stoechospermum marginatum, Gracilaria carticata* and *Gracilaria edulis* respectively. The green seaweeds like *Ulva lactuca* and *Ulva reticulata* contained 47±8.5 and 46.4±2.7mg of iron per 100 gm of dry sample respectively. Brown algae *Stoechospermum marginatum* contains

Table 2. Mineral composition of the selected seaweeds (mg/ 100gm of dry weight)

List of Mineral	Name of the selected edible seaweeds					
	Ulva lactuca	*Ulva reticulata*	*Stoechospermum marginatum*	*Acanthophora spicifera*	*Gracilaria corticata*	*Gracilaria edulis*
Calcium	2256±230.2	361.6±17.3	479.9±14.46	457.31±17.47	339.61±12.91	3603.28±362.2
Sodium	348±16.25	2291.6±45.3	1192.83±86.7	1391.8±259.4	1007.6±151.2	900±20
Potassium	152.7±8.35	4259±61.15	700±8.66	2633±30.5	871.3±30	6021±30.8
Iron	47±8.5	46.5±6.11	62.4±2.7	55.4±2.7	64.4±3.7	51.8±4.5

62.4±2.7mg of iron compared with the other selected seaweeds. The iron content of *Gracilaria corticata, Acanthophora spicifera* and *Gracilaria edulis* was found to be64.4±3.7 55.4±2.7 and 51.8±4.5 mg of iron respectively.

Discussion

Seaweeds are traditionally consumed in different part of the world. Recently human consumption of green algae (5%), brown algae (66.5%) and red algae (33%) is high in Asia. Asian countries, seaweeds are often consumed as marine vegetables (Soriano *et al.*, 2006). Seaweeds are rich in soluble dietary fibers, proteins, minerals, vitamins, antioxidants and polyunsaturated fatty acids, with a low calorific value (Mohamed *et al*., 2012). Santoso *et al.* (2006) also reported that seaweeds are the richest source of minerals. The macromolecules such Sodium (Na), Calcium (Ca), Potassium (K) and Magnesium (Mg) are among the minerals which are present in significant amounts in marine algae (Nisizawa, 2006). The seaweeds show great variation in nutrient content which are related to several environmental factors such as water, temperature, salinity, light and nutrients (Dawes, 1998). Most of the environmental parameters vary according to season and changes in ecological conditions that can stimulate or inhibit the biosynthesis of several nutrients.

The present study detected the elemental composition in the cell wall of the selected seaweeds using EDAX and reported that elements such as calcium, sodium, potassium, magnesium, chloride, silica were present in all the selected seaweeds. EDAX techniques can provide valuable inputs in determining the distribution of various elements over the seaweed surface (Figueria *et al*., 1999)

Hannan *et al.* (2015) reported that the chlorophycae species *Ulva lactuca* contain the most abundant amount of calcium. Manivanna *et al.* (2008) estimated the mineral composition of marine macro algae in Mandapam Coastal Regions and reported that *Ulva lactuca* contain 174.5 ± 13.15ppm of magnesium content. Kiuomars *et al.* (2012) reported that *Gracilaria corticata* contain 3.3mg of magnesium per 100gm. Chloride and Potassium, the macro elements maintain the osmotic gradients, ionic exchange and normal neural functions. Potassium is a very important mineral for the proper function of all cells, tissues, and organs in the human body. It has an important part in regulation of water balance of the body (Anderson *et al*., 2008).

Anantharaman *et al.* (2010) determined the trace metal and mineral composition of various seaweeds *Cladophora glomerata, Ulva reticulata, Halimeda macroloba, H.tuna, Dictyota dichotoma, Padina pavonica, Gracilaria crassa, Gelidiella acerosa, Hypnea musciformis* collected from Mandapam coastal regions, Southeast coast of India. Among these seaweeds, *Ulva reticulata* showed the maximum contents of mineral elements such as chromium, copper and magnesium. Murugaiyan and Narasimman (2013) investigated mineral content of six types of seaweeds from Gulf of Mannar coastal region and reported that copper and magnesium was observed maximum in *Ulva reticulata* with 125.09± 3.08 and 505.43± 41.96 respectively. Magnesium is the second most abundant intracellular cation and is involved in numerous enzymatic pathways including those of glucose metabolism. Intracellular magnesium plays a key role in the regulating insulin action, insulin mediated glucose uptake and vascular tone. Hypomagnesemia leads to insulin resistance and reduce functionality of various co- factors which may result in onset of diabetes (Biswajit and Seydouide, 2014).

Balakrishan *et al.*, (2013) investigated the elemental composition of *Gracilaria corticata* by EDAX and reported that 51.26, 46.75, 0.87, 0.38, 0.25, 0.39, 0.07, 0.02 percent of carbon, oxide, sulphur, magnesium, sodium, calcium, silica and potassium respectively were detected. Qualitative composition of elements such as calcium, sodium, potassium and iron were analyzed in the selected

seaweeds. Calcium content of the selected seaweed ranges from 361.6±17.3 to 3603.28±362.2. The study selected the three red seaweed *Acanthophora spicifera, Gracilaria carticata* and *Gracilaria edulis* and the study reported that the maximum amount of potassium present in *Gracilaria edulis* and the maximum amount of iron represents the *Gracilaria carticata*. Brown algae *Stoechospermum marginatum* contain a high amount of iron.

A high potassium diet may reduce the risk of hypertension and strokes (Folis, 1942; Elia et al., 2011). Ravi *et al.,* (2014) were collected various edible seaweeds during low tide periods from different sites along the Gujarat and Tamil Nadu coast, India. This study reported that *Gracilaria edulis* contain 13715±162.63, 2208±8.48, 672.6±27.71 and 58.3±0.42 mg/100g of potassium, sodium calcium and iron respectively. *Gracilaria corticata* showed potassium (7910±29.69), sodium (2680±87.64), calcium (310.65±34.011) and iron (21.65±2.05) in 100 gm of dried samples. Kiumars *et al*., (2012) carried out the proximate, fatty acid and mineral composition of seaweeds collected from Northern coast of Persian Gulf of Iran and reported that the potassium content of *Ulva lactuca* and *Gracilaria carticat*a contain 515.6±35.68 and 713.0±46.05 mg respectively. Ratana-arporn and Anong (2006) evaluated the nutrient content of *Caulerpa lentillifera* and *Ulva reticulata* was collected from Amphor Banlam, Petchburi Province in March and reported that *Ulva lactuca* had 1540 mg of potassium and 174.8mg of iron.

Mineral composition of *Ulva rigida* C. Agardh revealed balanced contents of Na and K (15.9 and 15.6 g/kg, respectively, a ratio near to 1 which, from a nutritional point of view, is of interest because intake of diets with a high Na/K ratio has been related to incidence of hypertension (Taboada *et al*., 2010). Calcium was the most important element, and accumulated in seaweeds at much higher levels than in terrestrial foodstuffs (Hanan *et al*., 2015). Among the minerals 4-7 percent of dry seaweeds contain calcium. Calcium present in the seaweeds in the form of calcium phosphate and it is more available than the form of calcium in milk. The present study reported all the selected seaweeds contain an appreciable amount of calcium, the range of 361±17.3 to 3603±362.2. The maximum amount of calcium detected in *Gracilaria edulis* and the minimum level of calcium present in Ulva reticulata.

Iron deficiency is the most prevalent cause of anemia with an estimated 50% of anemia cases attributable to iron deficiency. (McLean Cogswell *et al*., 2009). Seaweeds contain an abundant amount of iron and included in our daily diet would help to meet out the requirements and reduce the risk factor of iron deficiency. Bioavailability is a term used to describe the proportion of a nutrient in food that can be utilized for normal body functions (Dell, 1984; Watzke, 1998). The biological availability of minerals depends on the diet composition and is influenced by the levels and forms of present nutrient or non nutrient components, and finally by nutrient synergistic or antagonistic interactions (Watanabe *et al*., 1997). Thahira Banu and Uma Mageswari (2015) develop a chocolate incorporating with the seaweed Ulva reticulata and assessed the bioavailability. This study reported that the seaweed incorporated chocolate contained 56 mg of iron/ 100 gm and 11.80 mg of bioavailable iron.

Fayaz *et al.,* (2005) selected the seaweed *Kappaphycus alvarezzi* from the west coast of India, was analyzed for its chemical composition *K. alvarezzi* was found to be good source of minerals, viz 0.16% of calcium, 0.033% of iron, and 0.016% of zinc, which are essential for various vital biological activities. Bioavailability of iron by in vitro methods showed a higher efficiency in intestinal conditions than in stomach conditions. Ascorbic acid influenced higher bioavailability of iron. Venugopal, (2009), examined the iron bioavailability of iron in *Enteromorpho compressa* (Linnaeus) incorporated pokoda, a trational snack food in India and reported that the iron bioavailability was varied in acidic and alkaline condition. The acidic condition (pH 1.35) the bioavailability of iron in pokada with seaweed was found to be higher 27.1% than that in free seaweeds. Mineral contents of seaweeds vary according to species, wave exposure, seasons, environmental factors physiological factors, type of processing and method of mineralization (Mabeau and Fleurence, 1993). Endogenous and exogenous factors have participated on the variability of seaweeds mineral composition. The differences in mineral have in seaweeds tissues were observed also with the same seaweed species influenced by the stage of the living cycle and the age of the seaweed (Misurcova *et al*., 2011).

Conclusion

The result of the present study it may be concluded that the important elements like calcium. Potassium, sodium, magnesium, selenium and iron were detected in the selected seaweeds through SEM-EDAX. Sodium, potassium, calcium and iron were present in all the selected seaweeds in a considerable amount. The iron content of the selected seaweeds if

utilized in diet formulating would help to meet out the Daily Recommended Dietary Allowances. Seaweeds are abundantly present in marine environment and being used as human food and animal feed. On comparison of the terrestrial plants and animal foods, seaweeds contain higher amount of minerals. Micronutrient deficiency is a global threat and its leads by inadequate intake of minerals in our diet and the low bioavailability of minerals. Many researches worked on functional components of seaweed in various coastal areas and reported that the most of the edible seaweeds contain a considerable amount of macro minerals and a trace element, which is important for our daily bodily functions and meet out our daily need, compared the Recommended Dietary allowances. Seaweeds used as a part of our daily diet will improve the nutritional status of the population and help to eradicate the prevalent micronutrient deficiencies.

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