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PROXIMATE COMPOSITION OF TWO ULVA SPECIES FROM SOUTHEAST COAST OF TAMIL NADU, INDIA

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ABSTRACT

The investigation was carried out with the phycochemical constituents of green algae *Ulva fasciata* Delile and *Ulva intestinalis* L., from Southeast coastal regions of Tamil Nadu, India. The dry weight was found as 102.7 mg/g of fw., in *U. intestinalis* and 117.8 mg/g of fw., in *U. fasciata*. The ash content was 12.4 mg/g of fw., in *U. intestinalis* and 16.3 mg/g of fw., in *U. fasciata*. Total protein was 37.11% in *U. intestinalis* and 29.32% in *U. fasciata*. Total amino acid was 19.04% in *U. intestinalis* and 22.25% in *U. fasciata*.

KEYWORDS: Proximate, Phycochemical, *Ulva fasciata*, *Ulva intestinalis*, South East Coast of Tamil Nadu.

INTRODUCTION

Seaweeds are being utilized and used for consumption in many countries. Marine algae could be served as a source of vitamins, minerals, fatty acids, polyunsaturated fatty acids, lipids, free amino acids, and protein. Macroalgae can be classified as red algae brown algae or green algae depending on their nutrient and chemical composition. Seaweeds are highly rich in essential nutrients, in countries such as China, Japan, and Korea; they have been commonly utilized in human alimentation. Seaweeds are being consumed in Asia since ancient times. Further, marine seaweeds have been used 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] Macroalgae contain the maximum of 60% high amounts of carbohydrates, 10-48% of proteins and the minimum of 1- 3.5% of lipids with the 7-35% of variable content of mineral ash.[3] The oceanic bio-flora have become attractive alternatives for the production of the highly valuable biomass, then the terrestrial crops.[4] Bioactive compounds, food or biofuel are highly available in *Ulva* species.[5]

The *Ulva* species contain water-soluble polysaccharides and cellulose with sulphate. These marine algae have shown antihyperlipidemic, antiviral, anticoagulant, antitumor and antioxidant activities.[6-9] Lahaye and Axelos[10] reported that the algal genus *Ulva* has one of the most essential sources of gelling polysaccharide. Generally in seaweeds green and red contains higher protein content (15-35% dw.).

In general, from the critical review of literature, it has been observed that most studies on the nutrient contents of seaweeds have concerned fresh plants. Little is known of the effects of processing by drying or canning.

Word widely both the macroalgal and microalgal species are growing for foods and are increasingly being used for functional benefits beyond the traditional considerations of nutrition and health. There is substantial evidence for the health benefits of algal-derived food products, but there remain considerable challenges in quantifying these benefits, as well as possible adverse effects. The general factors such as the nutritional composition of algal species, geographical regions and seasons are substantially affecting the algal their dietary value. Quantitative fractions of seaweed foods are bioavailable to humans. Now, the process of understanding to know the algal nutritional and functional constituents interact in human metabolism. The effects of harvesting, storage, and food processing techniques dramatically influence the potential nutritive value of algal-derived foods.[11] To quantify the proximate composition of dry weigh, ash content, total protein and total amino acids of the selected marine green algal seaweeds.

MATERIALS AND METHODS

Fresh, matured, disease-free and healthy sample weighing approximately 1 kg of each seaweeds; two green algae *Ulva fasciata,* and *Ulva intestinalis* were selected seaweeds along the coast of southeast coast of Tamil Nadu, India during November 2017 to February – 2018, and they were washed thoroughly in seawater followed by tap water to remove the epiphytes and other extraneous materials. Then they were brought to the laboratory and stored at - 0 ^{0}C

till conducting further studies.

1. *Ulva fasciata* Delile

Plants light green, drying brownish, sometimes with a faint central stripe attached by a small disc, soon branched into several blades in a candelabra pattern, blades flat (not ruffled), narrowing to a point (elongate-lanceolate) blade edges usually smooth. Special requirements view the blades microscopically; 1. Blades consist of 2 sheets of cells, in short, curved rows, 2. The anatomical view at the base of blades has a thick sandwiched central mass of rhizoids growing from the surface cells. Usual Habitat widely distributed. The species is branched along with the blades and not just at the base, dries bright green, and has ruffled edges.

2. *Ulva intestinalis* L.

Ulva intestinalis Linnaeus is a green alga, known also as Gut Weed and Grass Kelp, in the division Chlorophyta, order Ulvales and family Ulvaceae, and occurs naturally worldwide. In general, this alga grows as a tube of 1-2 mm length, composed of irregularly arranged cells as a single layer. The alga thallus has smooth at the young stages, while later it is wrinkled and also changes its colour from dark green to light green or yellow-green. Branching occurs near the holdfast, which is small and narrow approximately 1mm.[12]

Ulva intestinalis[13] is found in many other parts of the world, including Europe and North America. It contains about 20% protein and has low in fat, sodium, high in iron and calcium. The vitamin B group content has generally higher than most vegetables, and while its vitamin A content is high, it is only half of that found in spinach. The marine algae could be lightly toasted to increase the flavor and powdered for use as a condiment on soups and foods, or it can be crushed into small pieces and used as a garnish. *Ulva intestinalis* is being used as animal feed, fodder, forage (bait/attractant, fishmeal, fodder/animal feed, forage and invertebrate food), fuels (biofuels), human food and beverage, food flour/starch, food additive, spices, culinary herbs and vegetable, biochemical, fertilizer, green manure, lipids and pesticide, and also the source of medicine and pharmaceutical.

PROXIMATE COMPOSITION ANALYSIS

DRY WEIGHT ANALYSIS

10gm of the frozen sample (W_1) of all the selected green algal seaweeds were weighed after blotting excess water kept in an oven at 45 ^{o} C till achieving constant weight (W_2). The dry weight of seaweed was calculated as W_2/W_1 x 100.

TOTAL ASH CONTENT[14]

Each alga weighing 1 gm of the dry sample obtained as described above (W_1) was kept at 550 ^{o} C for 4 h in a muffle furnace in silica crucibles. After charring, the content of crucibles was weighed (W_2) for ash content. Percentage of total ash of the sample was calculated as W_2/W_1 x 100.

PROTEIN[15]

Principle

The algal protein consist of certain tyrosine and tryptophan residues (amino acid) of the phenolic group which produce a blue-purple color complex with maximum absorption in the region of 660 nm with Folin – Ciocalteau reagent. Thus the intensity of the colour depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

Reagents

Alkaline copper reagents

Solution A: 2% sodium carbonate in 0.1N sodium hydroxide solution.

Solution B: 0.5% copper sulphate solution

Solution C: 1 % sodium potassium tartarate solution

50ml of solution A was mixed with 0.5ml of solution B and 1ml of solution C just before use.

Folin-Ciocalteau reagent solution

Hundred grams of sodium tungstate, 25 grams of sodium molybdate, 700ml of water, 50ml of 85% orthophosphoric acid and 100ml of concentrated hydrochloric acid were taken in a 1500ml round bottom flask. The mixture was refluxed gently for 10 hours. To this 150g of lithium sulphate, 50ml of water and a few drops of bromine were added and the mixture was boiled for 15 minutes to remove excess bromine. This was diluted 1:2 with distilled H_2O just before use.

Extraction

One gram of frozen crude carbohydrate sample was taken and homogenized in a prechilled mortar and pestle with 10 ml of ice-cold Tris buffer (0.1M pH-7). The algal extracts were separately centrifuged at 8000 rpm for 10 minutes and the supernatant was separately collected. 10% TCA (twice the volume) was added into the supernatant and kept overnight at 40°C. And then it was again centrifuged at 8000 rpm for 15 minutes and the pallet was pooled, dissolved in Tris buffer and it was finally used for the estimation of protein. The final

protein extract was made into 30 ml using 10 % TCA solution.

Procedure

An aliquot of 0.2ml of the sample was diluted to 5ml with distilled water and then added 2ml of alkaline copper sulphate reagent. Mixed the solutions well. This solution was incubated at room temperature for 10 minutes. Then 0.2ml of Folin- Ciocalteau reagent solution was added and incubated for 30 minutes. Optical density was read against the blank at 660nm and protein was calculated from the standard curve prepared by using BSA.

TOTAL AMINO ACIDS.[16]

Reagents

- 80% ethanol: 80 ml absolute alcohol was made up to 100 ml with glass distilled water
- 0.1N Na OH: 0.4g of NaOH pellets was dissolved and made up to 100 ml using glass distilled water.
- 1% Ninhydrin : 1 g of Ninhydrin was dissolved and made up to 100 ml with acetone
- Methyl red indicator n-Propanol

Extraction

One gram of frozen crude carbohydrate sample was taken and homogenized with 4 ml of 80% ethanol and pooled in a test tube. The homogenization was repeated at least thrice. The pooled extracts were centrifuged at 8000 rpm for 10 minutes and the supernatant was made up to 15 ml.

Estimation

To 1 ml of the above sample in a test tube, 1 drop of methyl red indicator was added and neutralized with 0.1 N NaOH. To this, 1 ml of Ninhydrin reagent was added and mixed thoroughly. The mouth of the test tubes was covered with glass beads and heated in a boiling water bath for 20 minutes. Then 5 ml of diluents (n-propanol: distilled water 1:1v/v) was added to the mixture while the samples were kept in the water bath. The sample containing test tubes were cooled under running tap water. The purple colour of the solution was read at 560 nm using spectrophotometer against a blank prepared without sample solution. The amino acid was calculated by using a standard graph prepared by varying concentration of glycine ranging from 10 –100 µg ml-1.

RESULTS AND DISCUSSION

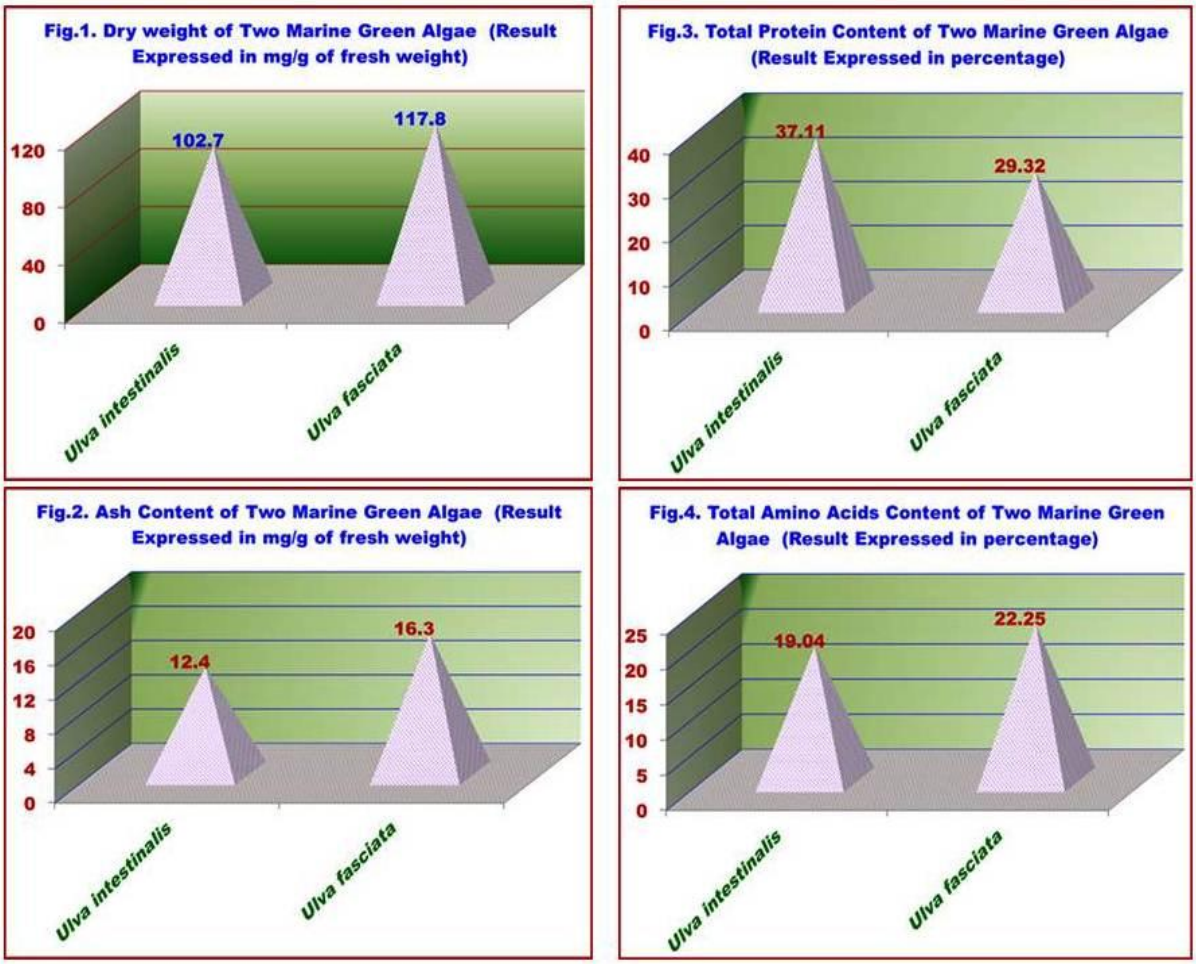
Seaweed is a sustainable natural resource for all the needs of human welfare like food, cosmetic, bioproducts, commercial, and industrial potential. About eight million tons of wet seaweeds are harvested annually worldwide.[13] Human consumption of green algae (5%), brown algae (66.5%) and red algae (33%) is being high in Asia, mainly Japan, China, and Korea.[17] Utilization of seaweeds for food has been extended to North America, South America, and Europe.[13] The different species consumed have great nutritional value as a source of proteins, carbohydrates, minerals, and vitamins. These seaweeds are harvested and utilized for a variety of purposes such as feed, fertilizer, bioactive compounds and as a source of raw material for industrial production of phycocolloids of commercial importance.[18,19] Biochemical composition of seaweeds varied according to species, seasons and habitats. Green algae contain more proteins than brown and red algae.[20] It has been reported that green algae contains 68- 88% water, 3-18% protein.[21]

In the present investigation, biochemical characteristics such as dry weight, ash content, total protein, and total amino acids, from the extract of two green seaweeds. Dry weights of seaweeds are presented in table 1 *U. fasciata* had the maximum of 117.8 ± 0.23 mg g-1 of fresh weight, and the next to *U. fasciata*, *U. intestinalis* had 102.7± 1.02 mg g-1 of fresh wt. (Table. 1;Fig. 1). Ash content of seaweeds is presented in table 2. Like dry weight, ash content also recorded maximum in *U. fasciata* 16.3±1.04 mg g-1 of fresh weight and the minimum quantity of ash content was 12.4±0.12 mg g-1 of fresh weight in *U. intestinalis* (Table -1; Fig.2).

Table - 1. Dry weight, Ash content, Total Protein and Total Amino acid of Two Marine green Algae

Parameters (Result Unit)	*Ulva intestinalis*	*Ulva fasciata*
Dry wt. (mg/g of fresh weight)	102.7 ± 1.02	117.8 ± 0.23
Ash content (mg/g of fresh weight)	12.4 ± 0.12	16.3 ± 1.04
Total Protein(percentage)	37.11 ± 0.03	29.32 ± 1.06
Total Amino acid (percentage)	19.04 ± 1.05	22.25 ± 0.22

Total protein content was estimated from the crude carbohydrate extracted in all the seaweeds. Total protein content varied in the individual species of seaweeds among the seaweeds investigated. Total protein of the selected seaweeds was presented in table 1, *Ulva intestinalis* had the maximum of 37.11±0.03% of the total protein and *Ulva fasciata* had 29.32±1.06%. (Table. 1; Fig. 3).



Total amino acid content was estimated from the crude protein estimated in all the seaweeds and the total amino acid content varied in the individual species among the seaweeds investigated. The maximum of total amino acid content was detected in *Ulva fasciata* 22.25±0.22% and the minimum quantity of amino acid was 19.04±1.05% in *Ulva intestinalis*. (Table -1; Fig.4). Sánchez- Machado *et al*.[22] reported ash content ranged from 19.07±0.61 to 34.00±0.11 g/100 g dry weight from *Himanthalia elongata, Laminaria ochroleuca, Undaria pinnatifida, Palmaria* sp. and *Porphyra* sp. In the present study, maximum of 117.8±0.23 mg g-1 dry wt. and a minimum of 102.7±1.02 mg g-1 dry wt. of dry weight content were recorded in *U. fasciata* and *U. intestinalis* which has higher than the previous report.[22] The pure protein content of seaweed products are being varied widely and also the available essential amino acids were tested in the seaweeds. Burtin[23] reported that the minimum quantity of protein content present in brown algal seaweeds whereas high the maximum of protein contents was recorded in red and green algal seaweeds. In the present study, the highest total protein content was recorded in *Ulva intestinalis* and *Ulva fasciata* which was similar to the above report.

CONCLUSION

In the present investigation, biochemical characteristics such as dry weight, ash content, total protein, and total amino acids, from the extract of two green seaweeds. The phycochemical analysis of *U. fasciata* and *U. intestinalis* showed the presence of total protein and total amino acids. Further studies are needed to determine the available potential compounds of the secondary metabolites and their biological applications for pharmacological effects.

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