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Potential of *Ulva linza* L. and *Caulerpa racemosa* var. *uvifera* seaweeds from Cox's Bazar, Bangladesh as sea vegetable

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Abstract

The nutritional quality parameters of leafy *Ulva linza* L. collected from the west coast of the Naf river estuary, Teknaf and grape-like *Caulerpa racemosa* var. *uvifera* collected from St. Martin's Island, Cox's Bazar, Bangladesh, were studied. Total protein content in *U. linza* (22.05 ± 1.73%) was found significantly ($p<0.05$) higher than *C. racemosa* var. *uvifera* (18.20 ± 1.50%) but with low crude lipids in the former; total amino acid content was also higher in the former, both having nearly the same amount of essential and non-essential amino acids; vitamin C content (about 2.5 mg/100 g) was nearly identical but β -carotene content was higher in the later (9.04 mg/100 g dry wt.). Crude fiber in *U. linza* was low (4.21 ± 0.43%) but high in *C. racemosa* var. *uvifera* (18.20 ± 1.50%). Other micronutrients were also ascertained. The nutritional quality parameters determined suggested that the "sea-salad" and "sea- grapes" can be used as sea-vegetables and food supplements.

Keywords: Food value; Nutrition; *Ulva linza* L.; *Caulerpa racemosa* var. *uvifera;* Sea salad; Sea grapes; Green seaweeds; Bangladesh

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Introduction

Seaweeds are widely consumed, especially in Asian countries as fresh, dried, or ingredients in prepared foods due to abundance of natural vitamins, minerals and palnt-based proteins (Fujiwara-Arasaki *et al.,* 1984; Nisizawa *et al.,* 2004; Zemke-White and Ohno, 1999;Fleurence 2016). The global population is going to increase by over one-third (2.3 billion people) by 2050, requiring an estimated 70% increased food production (Godfray *et al.,* 2010) whereseaweeds may serve as a food resource and nutrition supplements for humans. In addition they provide various environmental, ecologic, socio-economic benefits and services as food for marine biota, biofertilizer, biofuel, medicine and chiefly for economically important phycocolloids (Levering *et al.,* 1969; Chapman and Chapman, 1980; Abowei and Ezekiel, 2013). Green seaweeds can grow on mangrove plants, both rocky and sandy beaches. Many can tolerate low salinity and colonize in estuaries. Among some green seaweeds, *Ulva* sp., *Enteromorpha* sp., *Monostroma* sp., *Caulerpa* sp. and *Codium* sp. are commonly known as marine food sources (Lobban and Harrison, 1994). *U. lactuca* var. *rigida* was first called sea lettuce has been reported from bolders of Inani beach (Aziz *et al.,* 2008). Van Patten (2006) reported the use of *Ulva linza* as a garnish on "Seaweed salad in fancy restaurants". Aziz and Alfasane (2020) reported the occurrence of *Ulva linza* along the coast of Naf river at Noapara, Teknaf, and coast of Bakkhali river at Launch Ghat/Fishery Ghat, north of Cox's Bazar Town, Bangladesh and

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Caulerpa fergusonii forest from extreme south of the St. Martin's island (SMI). *C. racemosa* var. *uvifera* has long been described as an abundant green seaweed from the SMI (Islam, 1976) and subsequently by other researchers (Aziz, 2015). Guiry and Guiry (2020) reported *C. fergusonii* and *C. racemosa* var. *uvifera* nutritionally good and used as sea-vegetables or salad. As many as over 210 seaweed species occur along the 715 km long Bangladesh coast (Aziz and Alfasane, 2020) and their use as food, fertilizer, and ingredients (like phycocolloids) of industrial products may add to the economy of Bangladesh "The Blue Economy" (Aziz, 2015). The levels of intake of nutrients (essential elements) that on the basis of scientific knowledge, are to be adequate to meet the known nutrient needs of practically all healthy persons (Press, 1985). Under the context, Bangladesh Agricultural Research Institute (BARI), Gazipur, took a project on "Capacity building for conducting Adaptive Trails on Seaweed Cultivation in Coastal Areas" for cultivating nutritious seaweeds for food supplements, in industrial food products, and production of bio-fertilizers (hormones and organic fertilizer) (BARI, 2019).

Aziz *et al.* (2021) determined nutritional status of *Gracilaria-tenuistipitata* var. *liui* (red seaweed) from Nuniachara sand-flat, Moheshkhali Channel, Cox's Bazar and found it as a rich source of protein (25.55 ± 0.18%), β-carotene and mineral content, a moderate quantity of crude fiber, ash, moisture, carbohydrate, and total energy; low fats and heavy metals, and balanced amino acid profile (EAA 9.03 and Non-EAA 7.52%) and considered it as a nutritionally rich healthy sea vegetable. The present study aims to evaluate nutritional quality concerning amino acid profiles, vitamins, minerals, and heavy metal contents in the *U. linza* (sea salad) and *C. racemosa* var. *uvifera* (sea grapes) for their possible use as sea vegetables growing along estuaries and coasts of Bangladesh.

Materials and methods

Collection of samples

The seaweed samples were supplied by the project authority mentioned above for nutritional analysis. *U.linza* was collected from the west coast of Naf river near village Noapara (21º 06′ 48″ to 21º 06′ 58″ N and 92º 12′ 07″ to 92º 12′ 17″ E), Teknaf growing on semi-rotten branches of mangroves and along clay soil coast being submerged by tidal waters (Aziz and Alfasane, 2020). *C.racemosa* var. *uvifera* (was collected from the West coast of St. Martin's Island, Cox's Bazar, Bangladesh (20º 34′ 26″ to 20º 39′ 10″ N and 92º 18′ 51″ to 92º 18′ 51″ to 92º 20′ 17″ E) in March 2017, both were abundant from January to April. After collection, the two seaweeds were washed thoroughly, dried under direct sunlight and kept in zip-lock poly-bags for nutritional analyses using three replicates (few determinations were carried out with only one sample).

Chemical analysis

Determination of crude protein

Crude protein content of different seaweeds was determined by the Micro-Kjeldahl method (Guebel *et al.,* 1991).

Determination of crude lipid

Crude lipid content of seaweeds was determined by following the methods of Mehlenbacher (1960).

Determination of crude fiber

Crude fiber content was measured following the methods of AOAC (2000).

Determination of moisture

At room temperature (25°C), one gram of powdered sample was placed on the tray of the Automatic moisture determination machine, Model Chyo, IB-30, for 10-15 minutes, and the moisture content was recorded.

Determination of ash

Ash content was determined by following the methods of AOAC (2000).

Determination of carbohydrate

Carbohydrate percentage was determined by the method described by Edeogu *et al.* (2007).

Determination of available energy

The Atwater factor method (9.3 lipid) + (4.1 carbohydrates) + (4.1 protein) described by Eneche (1999), Chinma and Igyor (2007), and Nwabueze (2007) was used to calculate the available energy value. The protein, lipid, and carbohydrate proportions were multiplied by their physiological fuel values of 4.1, 9.3, and 4.1 kcal, respectively. The sum of the products was considered as available energy.

Determination of amino acids

Amino acid analysis was conducted using the Shimadzu HPLC amino acid analysis system (Anonymous, 1993).

Determination of vitamins

Determination of β-carotene content was carried out following column chromatography and quantification by visible spectroscopy according to the method of AOAC (1990). Vitamin C was measured by HPLC methods of Lakshanasomya (1998), and Knelfel and Sommer (1985).

Determination of minerals

The mineral contents (Ca, Mg, Fe, Cu, and P) were determined by the methods described in the Manual of Laboratory Techniques (National Research Council (US), 2006).

Determination of heavy metals

This analysis was carried out by Atomic Absorption Spectrometry (AAS). The quantities of chemical elements present in environmental samples were determined by measuring the absorbed radiation of the chemical element of interest. This was done by reading the spectra produced when the sample is excited by radiation (García and Báez, 2012). Arsenic was determined as total arsenic.

Statistical analysis

Statistical analyses were carried out by using Statistical Package for Social Science (SPSS) for Windows version 15.0. All data were subjected to ANOVA test. The results obtained in the present study are reported as mean values (obtained from the three replications) ± standard deviation (SD). The significant differences between mean values were analyzed by the Duncan multiple range test at a significance level of $p<0.05$ and $p<0.005$.

Results and discussion

Nutrient composition

The biochemical compositions of *U. linza* and *C. racemosa* var. *uvifera*, including crude protein, crude lipids, crude fiber, moisture, ash, carbohydrate, energy, amino acids, and vitamins, were determined (on a dry weight basis) and compared to smereportd species (Tables I-III). The crude protein content of *U. linza* was 22.05 ± 1.73%, which was comparable to *U. reticulata* but higher than *U. lactuca* shown in Table I. *Gracilariatenuistipitata* var. *liui* (red seaweed) from Nuniachara sand-flat, Moheshkhali Channel, Cox's Bazar was reported to be a rich source of crude protein (25.55 ± 0.18%) (Aziz *et al.*, 2021). Furthermore, the crude lipid content in both species was identical (1.09 ± 0.18% and 1.24 ± 025%, respectively) and significantly lower than the *C. racemosa* (7.64 %). Nonetheless, the crude fiber content of *U. linza* (4.21 0.43%) was significantly ($p<0.005$) lower than that of *C. racemosa* var. *uvifera* (18.20 ± 1.50%) but comparable to that of *U. lactuca* (Table I). Moisture content in *U. linza* (13.70 ± 1.55%) was slightly higher than *C. racemosa* var. *uvifera* (10.45 ± 1.72%) and the value usually varied depending on drying and storing conditions (Mwalugha *et al.* 2015). Among the reported species, *U. linza* had the highest amount of ash (21.09 ± 0.35 %), indicating that the seaweed contains a large amount of total minerals (Table I). The total carbohydrate content of *U. linza* and *C. racemosa* var. *uvifera* was 37.86 ±2.25 % and 41.70 ±2.61 %, respectively, which was significantly lower/higher than other species reported elsewhere (Table I). The energy content of *U. linza* was 255.77± 17.99 kcal/100 g, which was very similar and significantly ($p<0.005$) higher than the two species previously reported.

Amino acid profile

The amino acid profiles of *U. linza* and *C. racemosa* var. *unifera* revealed a total of 13 amino acids were found in the two species, 9 of which were essential amino acids (EAA) and 4 of which were non-essential amino acids (NEAA), and both species had nearly identical amounts of total amino acid 13.29 and 12.58 %, respectively (Table II). Lysine was present in higher percentage in *U. linza* among EAA, followed by leucine, arginine, and so on, whereas glutamate was present in higher percentage in NEAA followed by alanine, aspartate, and serine. Both *U. linza* and *C. racemosa* var. *unifera* contain significantly more essential and non-essential amino acids ($p<0.05$) than *U. lactuca* and *U. reticulata* as previously reported (Table II). When compared to *Gracilariatenuistipitata* var. *liui*, the amino acid profile (EAA 9.03 and Non-EAA 7.52%) was lower (Aziz *et al*., 2021).

Vitamins and minerals

The β-carotene content of *U. linza* was found to be very low (1.68 mg/100 g) when compared to *C. racemosa* var. *uvifera* (9.04 mg/100 g) in a single sample. Vitamin C content (2.0 mg/100g) was very low in both species (Fig. 1) as determined by a single sample. According to Sarojini and Sarma (1999), the vitamin C content of Chlorophyceae ranges from 8.9 to 9.5 mg/100g. The current species' low vitamin C content could be attributed to drying processes and preservation techniques (Igwemmar *et al.,* 2013; Santos and Silva, 2008). The β-carotene content in *Gracilariatenuistipitata* var. *liui,* was reported to be higher (11.54 ± 1.20 mg/100g) than both of the green seaweeds (Aziz *et al*., 2021).

Among the minerals studied, Ca (381.33mg/100g) and P (576.55 mg/100 g) contents were high in *C. racemosa* var. *uvifera*, while only P (530.96 mg/100 g) was higher in *U. linza* (Fig. 2). *U. linza* (88.68 mg/100g) had a higher Fe

Table I. Nurtitional content (% dry wt.) of of *U. linza* and *C. racemosa* var. *uvifera* and comparison with other reported green seaweed species

Name of seaweeds	Crude protein	Crude lipids	Crude fiber	Moisture	Total ash	Total carbo - hydrate	Energy (kcal/100g)	References
U. linza	[a]22.05 ± 1.73	1.09 ± 0.18	4.21 ± 0.43	[c]13.70 ± 1.55	21.09 ± 0.35	37.86 ± 2.25	[d]255.77 ± 17.99	Present study
C. racemosa var. *uvifera*	18.20 ± 0.50	1.24 ± 0.25	[b]18.20 ± 1.50	[c]10.45 ± 1.72	13.44 ± 0.46	41.70 ± 2.61	[d]257.12 ±19.18	Present study
Reported Species								
U. reticulata	21.06 ± 0.42	1.34 ± 0.09	NR	22.51 ± 0.97	17.58 ± 2.00	25.57 ± 1.22	[e]207.75 ± 1.15	Ratana-arporn and Chirapart, 2006
C. lentillifera	12.49 ± 0.30	1.91 ± 0.06	3.17 ± 0.21	25.31 ± 1.15	24.21 ± 1.70	59.27 ± 2.21	[f]311.98 ± 10.85	Ratana-arporn and Chirapart, 2006
U. lactuca	14.99 ± 0.54	1.65 ± 0.17	4.84 ± 0.33	NR	12.37 ± 0.72	23.07 ±00.91	[e]175.24 ±00.81	Mwalugha *et al.,* 2015
C. racemosa	19.72 ± 0.77	7.65 ± 1.19	12.38 ± 0.10	NR	12.15 ±00.46	48.97 ± 1.22	NR	Bhuiyan *et al,* 22016

n=3; Mean ± SD, SD= Standard deviation, NR- Not reported.[a]P<0.05, [b]p<0.005 significantly higher compared to other species, [c]p<0.001 significantly lower compared to other species.[d]P<0.005 significantly higher compared to other species [e]p<0.005 significantly lower than f

Table II. Amino acid profile (% dry wt.) of *U. linza and C. racemosa* var. *unifera* and comparison with other two reported green seaweed species

Sl. No.	Name of amino acid	*U. linza*	*C.racemosa* var. *uvifera*	*U. lactuca* (Kumar and and Kaladhara, 2007)	*U. reticulata* (Ishakani *et al.,* 2017)
1.	Arginine	0.89 ±0.02*	0.78 ±0.02*	0.89	0.93
2.	Histidine	0.35 ±1.05	0.51 ±0.01	0.31	0.50
3.	Iso-Leucine	0.79 ±0,02	0.59 ±1,05	0.38	0.51
4.	Leucine	[a]1.24±0.01	[a]1.23 ±0.04	[c] 0.55	[c]0.89
5.	Lysine	[a]1.27 ±0.02	[a]1.12 ±0.03	[c] 0.46	[c]0.52
6.	Methionine	0.50±0.02	0.27±0.05	0.19	0.48
7.	Threonine	0.66±0.01	[a]1.05±0.02	0.99	0.00
8.	Tyrosine	0.43 ±0.03	0.34 ±0.02	0.39	0.00
9.	Valine	0.77±0.05	[a]1.09±0.03	0.66	0.57
	Total EAA	6.90	6.98	4.82	4.40
10.	Alanine	[a]1.38 ±0.03	0.94 ±0.02	0.85	0.50
11.	Aspartate	[a]1.72±0.02	[a]1.17±0.03	1.59	1.08
12.	Glutamate	[b]2.40±0.04	[b] 2.35±1.05	1.40	1.12
13.	Glycine	0.89± 0.03	[a] 1.14±1.04	0.71	1.03
	Total Non -EAA	6.39	5.60	4.55	3.79
	Total AA	13.29	12.58	9.37	8.19

n=3, *Mean± SD, SD= Standard deviation, EAA= essential amino acid, Non-EAA= non-essential amino acid, [a]p<0.05,[b]p<0.05 significantly different compared to other amino acids. [c] Data based on one sample

Table III. Heavy metal content (mg/kg dry wt.) in U. linzaandC. racemosa var. uvifera and comparison with other reported green seaweed species

Name of seaweeds	Pb	As	Cr	Cd	References
U. linza	[a]0.234±0.02*	[c]0.016±0.02*	0.041 ±0.02*	0.029±0.02*	Present study
C. racemosavar. uvifera	[a]0.193±0.02	[c]0.016±0.02	0.112±0.02	0.026±0.02	Present study
Reported Species by others					
U. lactuca	0.180	NR	0.140	0.018	Manivannan *et al.*, 2008
C. racemosa	[b]4.450	[d]1.170	0.029	0.030	Komalavalli and Lalitha, 2015
U. stenophylla	[b]1.830	[d]1.880	NR	NR	Smith *et al*., 2010
C. lentillifera	0.160	[d]1.060	NR	NR	Paul *et al.,* 2013

n=3, *Mean± SD, SD= Standard deviation, NR= Not reported, [a]significantly different (P<0.05) compared to b

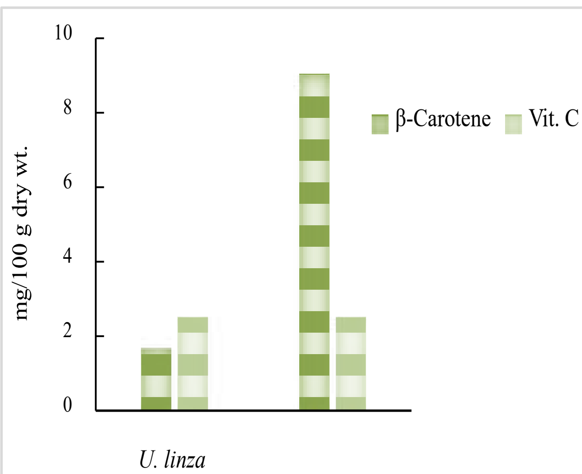

Fig. 1. Vitamines in U. linza and C. racemosa var. uvifera

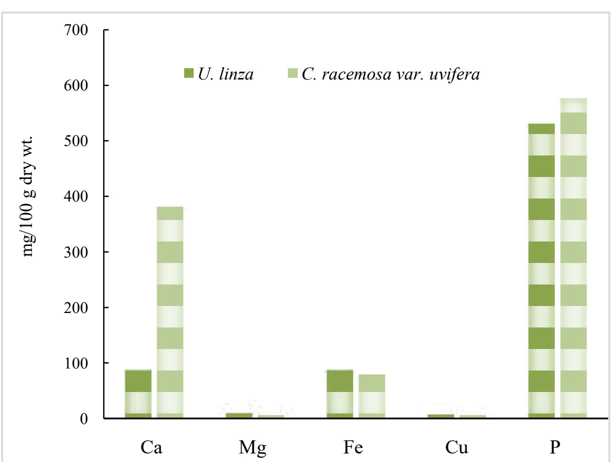

Fig. 2. Metals in U. linzaand C. racemosa var. uvifera

content than *C. racemosa* var. *uvifera* (79.40 mg/100g). In contrast, the phosphorus (P) content of both seaweeds was highest. Mg content in *U. linza* was very low (4.88 mg/100g) and absent in *C. racemosa* var. *uvifera*. Copper (Cu) content was extremely low in both seaweeds (Fig. 2).

Heavy metals

The lead content of *U. linza* was 0.2343 mg/kg (permissible limit 0.1-0.3 mg/kg by FSAI, 2009; and 0.5 mg/kg in the United States by FDA, 2021), which was significantly ($p<0.05$) higher than *C. racemosa* var. *uvifera* (0.1930.02 mg/kg), but lower than *C. racemosa* and *U. stenophylla* (Table III). The total arsenic content of *U. linza* and *C. racemosa* var. *uvifera* was similar (0.0163 mg/kg), much lower than the permissible limit of 0.3 mg/kg in the United States (FDA, 2021) and significantly ($p<0.05$) lower than that of *C. racemosa*, *U. stenophylla*, and *C. lentillifera* (0.106mg/kg). Chromium and Cadmium content in *U. linza* and *C. racemosa* var. *uvifera* were 0.0410 and 0.0192 mg/kg, respectively (permissible limits in USA are 0.2 mg/kg and 0.1 mg/kg, respectively) (FDA, 2021). In contrast, French legislation has set a maximum level of Cd in dried seaweeds at 0.5mg/kg. Aziz *et al*. (2021) found that all heavy metals in *Gracilariatenuistipitata* var. *liui* were below the permissible limits. Although element analysis in seaweeds from China, Japan, and Korea has been reported, but surprisingly no such permissible limit for Asia has yet to be established (Chen *et al.,* 2018; Dawczynski *et al.,* 2007; Khan *et al.,* 2015).

Considering the nutritional values of seaweeds, in Asia (China in particular) they are being used as seafood since ancient time. Moreover, in Europe, USA, and other western countries, seafoods are being sold in Supershops (Fleurence, 2016; Barba 2017; Granato *et al.,* 2020).

In diet planning, food should be composed of a variety of items derived from various groups, that are acceptable and palatable ensuring consumption. The levels of intake of essential nutrient elements deemed adequate by the Food and Nutrition Board based on scientific evidence to meet the known nutrient needs of virtually all healthy people (Press, 1985). However, physiological and social values of introduced food items are difficult to quantify.

With global warming Bangladesh's coastal areas are becoming more vulnerable to natural disasters that affect crop-field soil by increasing salinity, thus crop selection and cropping patterns need to be altered when seaweeds appear to be promising crops adapting to the changing coastal environment ensuring food security (Aziz *et al.*, 2021).

Conclusion

The study revealed that *U. linza* and *C. racemosa* var. *uvifera* collected from Naf river bank and St. Martin's Island are high in protein, fiber, ash, carbohydrate, energy, minerals, rich sources of macro and micronutrients, low lipid content, and a balanced amino acid profile thus considered as nutritious seafoods.Furthermore, the two seaweeds are arsenic-free and have low concentrations of Cr, Cd, and Pb, thus considered as safe sea vegetables, food ingredients and supplements. The nutritional status has been thoroughly addressed, which is expected to promote and assist industry policymakers, consumers and dietitians for future use. Research is in progress for growing seaweeds in large scale along Bangladesh coast for adapting in terms of crop production to the changing coastal environment.

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