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Proximate Chemical Composition of Sea Grapes *Caulerpa racemosa* (J. Agardh, 1873) Collected from a Sub-Tropical Coast

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Abstract

Background: Nutritional fact study has prime importance to make the species edible and commercially viable to the consumers. Proximate chemical composition and amino acid profile were investigated to understand the nutritional value and protein quality of an edible algae Caulerpa racemosa.

Methods: Samples were collected randomly by hand from the intertidal zone of the sub-tropical coastal Island St. Martin's Island from February 2013 to May 2014. Samples were preserved using standard methods for chemical analysis. Proximate composition was determined using standard methods, Kjeldahl method for protein, Soxhlet method for crude lipid, H_2SO_4 (0.3 N) and NaOH (0.5 N) for dietary fibre, muffle furnace method for moisture content, ion-exchange chromatography for amino acid and statistical package used for validating the data.

Results: The result of the study reveals that *C. racemosa* contains higher amount of proteins (19.72±0.77%), crude lipid (7.65±1.19%) and fibre (11.51±1.32%) compared to other green and brown algae. The higher concentration of aspartic acid (12.7±0.2%) and glutamic acid (9.2±0.7%) were observed in *C. racemosa*, while histidine (2.6±0.7%), methionine (1.4±0.4%) and tyrosine (3.8±0.2%) were the limiting amino acids. Lysine (6.6±0.2%), leusine (6.9±0.6%), glycine (6.5±0.4%), arginine (6.4±0.3%), alanine (7.6±0.6%) and threonine (6.2±0.5%) were obtained at a higher percentage of total amino acids.

Conclusion: This study suggests that *C. racemosa* could be potentially used as a nutritious and functional food item for human consumption. Further studies on this edible species should be focused on fatty acid composition, vitamins, non-starch polysaccharide constituents, trace elements and sensory perceptions in order to depict safer and versatile utilization.

Keywords Caulerpa racemosa; Green seaweed; Proximate chemical composition

Introduction

Asian people have a long tradition of consuming different types of seaweeds in their diet [1]. Nutritional compositions of a number of seaweeds have demonstrated that seaweeds are great source of protein, fatty acids, dietary fibre and some essential minerals [2-6]. Utilization of different types of seaweeds not only limited to human diet, but also used as an ingredient for animal and poultry feed [3,7]. Using of seaweed polymers as a thickening and gelling agents is of growing interest to the food and pharmaceutical industries in Western countries [1,8]. Recently, due to having higher nutritional constituents, the demand of some edible seaweed has increased in Europe, North America, and South America [9]. Some trace elements are rare in terrestrial plants but they are rich in seaweeds [8,10]. Some seaweed species have antibacterial, antiviral and antifungal properties, which are used for fungicide, herbicides and various industrial applications [11,12]. Some compounds of seaweeds control high blood pressure, level of cholesterol, and prevent cardiac arrhythmia. These can also be used as remedy for rheumatism, diarrhoea, and for controlling the growth of tumour [4].

From 250 commercially utilized seaweeds, only 150 seaweed species are edible and widely consumed as fresh, dried or food ingredients [11]. In Bangladesh, total 137 species of seaweeds (e.g. green, brown and red algae) were recorded from the Saint Martin's Island, Chittagong coast, Cox's Bazar coast and Sundarban mangrove area, Khulna [13]. Among them, *C. racemosa* is very common edible green seaweeds and growing abundantly at St. Martin's Island [7]. Characteristically, *C. racemosa* (commonly known as sea grapes) is green algae, growing on the rocky or hard substratum of intertidal water [1,7]. It is so nice in appearance, if it is looked through the water;

it seems grapes are lying down in the water. In the St. Martin's Island, *C. racemosa* is found in the lagoons situated along the western side of Cheradia (a separated islet). Local people of St. Martin's Island like to consume globular of raw *C. racemosa*. But, *C. racemosa* has yet been got much acceptance to the people of Bangladesh [7].

Several environmental factors such as water temperature, salinity, light and nutrients can influence the nutritional contents of seaweeds [10,14]. A few numbers of studies on *C. racemosa* have been reported especially on general morphology, abundance, distribution, standing stock [4,7] and micronutrients and trace elements [15]. Only a few studies have documented the nutritional composition of some other edible seaweeds [21, 22] in the sub-tropical coastal area of Bangladesh but proximate composition and amino acid profile of *C. racemosa* is very scantly and nutritional data on *C. racemosa* is not yet available in the literature. It has always been realized that nutritional fact study has prime importance to make the species edible and commercially viable to the consumers [7,16]. The main objective of this study was to determine the proximate chemical composition (protein, carbohydrate, lipids, fibre and ash) of *C. racemosa* from the sub-tropical coastal Island St. Martin's, to evaluate the potential nutritive value of this green seaweed. Moreover, amino acid profile was also investigated in order to know more comprehensive nutrient information about C. racemosa since amino acids are necessary for the dietary needs of humans or other animals.

Material and Methods

Study area

The green seaweed *C. racemosa* was collected from a sub-tropical coastal Island named St. Martin's Island from February 2013 to May 2014. The St. Martin's Island is situated in the extreme South-eastern corner of Bangladesh where coral reefs grow naturally and abundantly. Geographically, it is located on the southern-most tip of the country, roughly between 20° 34′ - 20° 39′ N and 92° 18′ - 92° 21′ E and separated by a channel of about 8 km from the mainland. The average turbidity (Secchi disc) of in-shore waters of St. Martin's Island ranges from 1.5 to 8.0 m. Water temperature and salinity fluctuated from 22-29°C [17] and 21.0-33.5 PSU [7], respectively, throughout the year. The Island has four coasts namely Western, Eastern, Southern and Northern coast. Naturally, seaweeds are not found in all the coasts, but the Western coast is suitable for proper development and growth of seaweed, which was selected for study (Figure 1). Three sampling stations at 200 m of distance in the Western part of the Island were selected. Seaweed *C. racemosa* is available throughout the year in this selected location.

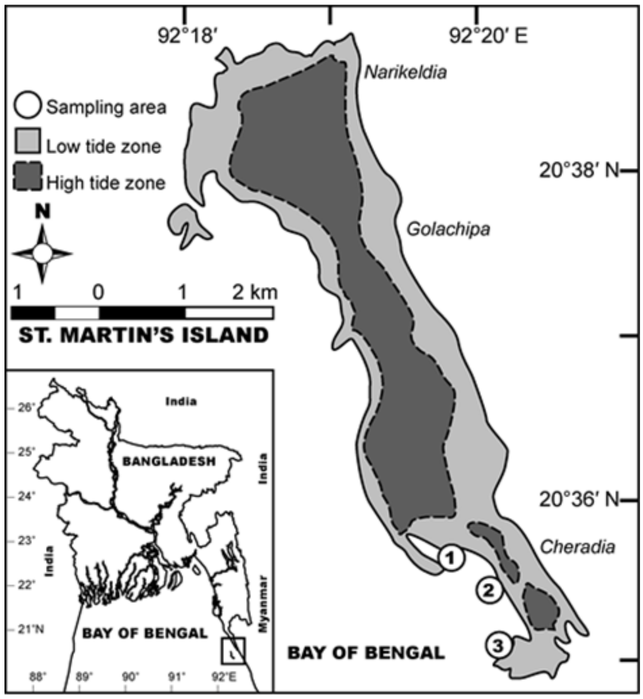

Figure 1: Sampling location in the St. Martin's Island, Bangladesh.

Seaweed collection and preservation

During low tide, *C. racemosa* was collected randomly by hand from the intertidal zone. Some floating seaweeds were collected from the surface water using a bamboo stick. Fresh samples were firstly washed under running seawater, then rinsed and gently washed again with distilled water. Samples were taken into plastic jars and brought back to the laboratory with ice. In the laboratory, the seaweed samples were freeze-dried in a Terroni Fauvel (model LH-1500) device for 3 days, and then the dried seaweeds were powdered manually using a mortar and pestle. Until the chemical analysis, the powder seaweed was stored in desiccators at a room temperature.

Proximate composition analysis

The proximate chemical compositions of *C. racemosa* were determined according to the standard method [18]. Protein content of *C. racemosa* was analysed by Kjeldahl method, where a conversion factor of 6.25 has been used to convert total nitrogen into the crude protein. Carbohydrate was determined simply by subtracting the mean percentage of protein, lipid, fibre, moisture and ash content from 100 [19]. Crude lipid of *C. racemosa* was extracted from the seaweed powder with petroleum ether in a Soxhlet extractor [20]. After completion of the extraction process, petroleum ether was evaporated and the residue was dried to a constant weight at 105°C. To measure the dietary fibre, 2 g of seaweed samples were previously boiled with diluted H_2SO_4 (0.3 N) and then the mixture was filtered and washed with 200 ml of boiling water and NaOH (0.5 N), respectively according to Siddique et al. [21]. The residue of the mixture was re-extracted and washed again with boiling water and acetone. Finally, the residual was dried at 105°C for 3 h to constant weight. The moisture content of *C. racemosa* was determined by drying the seaweed samples at 105°C to constant weight. Then the moisture content was calculated by subtracting the final weight from the initial weight of sample. The ash content was obtained by calcinations in a muffle furnace at 550°C for 4 h [21,22].

Amino acid analysis

Amino acid analysis was carried out by ion-exchange chromatography followed Siddique et al. [21]. A sub-sample (containing 5.0 mg of protein) was taken for acid hydrolysis. 1 ml of HCl acid (6 N) was taken with the sub-sample in a vacuum-sealed hydrolysis vials and heated 22 h at the temperature of 110°C. Norleucine was added to the HCl acid as an internal standard. Few amino acids such as tryptophan, cystine and cysteine were completely lost during the acid hydrolysis process. The tubes were cooled after hydrolysis process and placed in desiccators with some NaOH pellets for 5-6 days. Before analysing the amino acids, the residue was dissolved in a suitable volume of a sample dilution of Na-S buffer with pH 2.2. Then the solution was filtered through a 0.22 µm Millipore membrane and prepared for amino acids analysis. A Beckman instrument (model 7300) has been used for the ion-exchange chromatography. During the acid hydrolysis process, some ammonia content usually comes from the degradation of amino acids [23,24]. Therefore, the ammonia content was included in the calculation of protein nitrogen retrieval.

Statistical analysis

All data were expressed in terms of mean ± standard deviation. To find out the mean percentage and standard deviation, Statistical Package for Social Science (SPSS Version 16.0 for windows) was used in this study.

Results

The mean percentages (dry weight basis) of protein, crude lipid, carbohydrates, fibre, ash and moisture in *C. racemosa* were not varied widely among the sampling stations (Table 1). The proximate compositions of *C. racemosa* were found: protein 19.72±0.77%, crude lipid 7.65±1.19%, carbohydrates 48.97±1.22%, fibre 11.51±1.32%, ash 12.15±0.46% and moisture 15.37±0.72% from St. Martin Island.

Sampling Stations	Protein (%)	Crude lipid (%)	Carbohydrates (%)	Fibre (%)	Ash (%)	Moisture (%)
I	20.26±1.02	8.92±0.54	47.74±2.21	11.23±0.76	11.85±0.18	14.68±0.52
II	18.84±0.41	6.54±0.12	48.99±0.42	12.95±1.05	12.68±0.36	16.12±0.24
III	20.06±0.86	7.49±0.61	50.18±1.04	10.35±0.34	11.92±0.74	15.32±0.98
Mean ± SD	19.72±0.77	7.65±1.19	48.97±1.22	11.51±1.32	12.15±0.46	15.37±0.72

Table 1: Proximate chemical composition of *C. racemosa* (dry weight basis, n = 3) collected from St. Martin's Island, Bangladesh.

Most of the essential amino acids i.e., arginine, histidine, isoleucine, lysine, leucine, methionine, phenylalanine, tyrosine, threonine and valine and six non-essential amino acids (alanine, aspartic acid, glutamic acid, glycine, proline and serine) were found in *C. racemosa*. The ratio of essential amino acids to non-essential amino acids in this study was found 0.94. Beside the higher concentration of aspartic acid (12.7±0.2%) and glutamic acid (9.2±0.7%), some other essential and non-essential amino acids such as lysine (6.6±0.2%), leusine (6.9±0.6%), glycine (6.5±0.4%), arginine (6.4±0.3%), alanine (7.6±0.6%) and threonine (6.2±0.5%) were obtained at a higher percentage of total amino acids (Table 2).

Amino acid	Caulerpa racemose (Present study)	Caulerpa fastigiata [c]	Codium decorticatum[c]	Codium spongiosum [c]	Codium taylorii [c]	Ulva fasciata [c]
Arginine[a]	6.4 ± 0.3	5.5 ± 0.6	5.2 ± 0.3	4.0 ± 0.4	3.8 ± 0.2	5.6 ± 0.5
Histidine[a]	2.6 ± 0.7	2.2 ± 0.3	3.5 ± 0.3	2.3 ± 0.4	2.7 ± 0.9	2.4 ± 0.4
Isoleucine[a]	5.8 ± 0.3	4.0 ± 0.0	4.0 ± 0.4	4.4 ± 0.5	4.4 ± 0.3	3.9 ± 0.5
Lysine[a]	6.6 ± 0.2	7.1 ± 0.1	6.4 ± 0.2	6.8 ± 0.3	7.5 ± 0.1	5.1 ± 0.3
Leucine[a]	6.9 ± 0.6	8.7 ± 0.3	8.5 ± 0.7	8.4 ± 0.6	8.2 ± 0.2	7.6 ± 0.5
Methionine[a]	1.4 ± 0.4	1.5 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	2.0 ± 0.2	0.9 ± 0.0
Phenylalanine[a]	5.0 ± 0.6	6.6 ± 0.2	5.1 ± 0.7	5.4 ± 0.5	6.1 ± 0.3	5.1 ± 0.2
Tyrosine[a]	3.8 ± 0.2	3.9 ± 0.2	2.3 ± 0.3	2.3 ± 0.1	2.8 ± 0.2	3.3 ± 0.5
Threonine[a]	6.2 ± 0.5	4.8 ± 0.1	6.1 ± 0.2	5.4 ± 0.3	4.5 ± 0.2	5.1 ± 0.3
Valine[a]	5.1 ± 0.3	6.1 ± 0.1	6.3 ± 0.1	6.6 ± 0.5	6.9 ± 0.5	5.7 ± 0.6
Alanine[b]	7.6 ± 0.6	6.2 ± 0.1	8.9 ± 0.7	8.1 ± 0.7	6.7 ± 0.4	8.5 ± 0.9
Aspartic acid[b]	9.2 ± 0.7	10.1 ± 0.2	10.8 ± 0.3	12.0 ± 0.6	10.6 ± 0.4	13.0 ± 0.6

Glutamic acid[b]	12.7 ± 0.2	10.7 ± 0.1	12.0 ± 1.1	14.1 ± 0.9	11.3 ± 0.4	12.6 ± 0.1
Glycine[b]	6.5 ± 0.4	7.1 ± 0.6	7.3 ± 0.7	6.1 ± 0.3	5.4 ± 0.2	6.5 ± 0.4
Proline[b]	5.1 ± 0.4	7.7 ± 0.4	4.9 ± 0.6	4.6 ± 0.2	7.9 ± 0.8	4.6 ± 0.2
Serine[b]	5.8 ± 0.4	6.2 ± 0.1	5.2 ± 0.3	5.3 ± 0.3	5.8 ± 0.6	5.8 ± 0.8
Ammonia	1.4 ± 0.4	1.0 ± 0.2	1.8 ± 0.2	1.7 ± 0.1	1.1 ± 0.1	1.8 ± 0.2
Total	98.1	98.4	97.2	96.6	96.6	95.7

Note: [a] EAA (essential amino acids); b Non-EAA (non-essential amino acids), c Lourenco et al. [33].

Table 2: Total amino acid compositions of *C. racemosa* and some other species of green algae; results are expressed as percentage of amino acid/100 mg of algal protein and all the values indicate the mean of three sampling stations with three replicates ± SD (n = 9).

Histidine (2.6±0.7%), methionine (1.4±0.4%) and tyrosine (3.8±0.2%) were present in lower amounts compared to other amino acids. Results are expressed as percentage of amino acid/100 mg of algal protein and represent the real recovery of amino acids after analysis. Concentrations of ammonia correspond to nitrogen recovered from some amino acids destroyed during acid hydrolysis. Data on tryptophan were not included in this work since this amino acid was destroyed during acid hydrolysis.

Discussion

The green seaweed *C. racemosa* was found to contain higher amount of protein, crude lipid and fibre content compared to other green and brown seaweed reported by various authors (Table 3).

Species	Protein	Lipid	Carbohydrate	Fibre	Ash	Moisture	Reference
Caulerpa racemosa [a]	19.72	7.65	48.97	11.51	12.15	15.37	Present study
Caulerpa lentillifera [a]	12.49	0.86	59.27	3.17	24.21	25.31	[16]
Enteromorpha sp. [a]	9.45	-	-	-	36.38	9.00	[38]
Ulva reticulate [a]	21.06	0.75	55.77	4.84	17.58	22.51	[16]
Ulva rigida [a]	6.40	0.30	18.10	-	52.00	-	[30]
Ulva lactuca [a]	7.06	1.64	14.60	-	55.40	10.60	[6]
Ulva pertusa [a]	15.4	4.8	-	-	27.2	6.0	[36]
Ulva intestinalis [a]	17.9	8.0	-	-	27.6	6.3	[36]
Sargassum filipendula [b]	8.72	-	3.73	6.57	44.29	-	[37]
Sargassum vulgare [b]	15.76	0.45	67.80	7.73	14.20	14.66	[26]
Gracilaria changgi [c]	6.9	3.3	-	24.7	22.7	-	[27]
Gracilaria cornea [c]	5.47	-	36.29	5.21	29.06	-	[37]
Gracilaria cervicornis [c]	22.96	0.43	63.12	5.65	7.72	14.33	[26]
Gelidium pristoides [c]	11.80	0.90	43.10	-	14.00	-	[30]
Hypnea japonica [c]	19.00	1.42	4.28	-	-	9.95	[6]
Porphyra tenera [c]	34.20	0.70	40.70	4.80	8.70	-	[25]

Note: [a] Green algae; [b] Brown algae; [c] Red algae

Table 3: Proximate chemical analysis of different seaweed species reported by various authors.

A few number of studies showed that red seaweed contains higher amount of protein and dietary fibre than some other green and brown seaweed [25,26]. Although, the mean percentage of protein obtained from *Gracilaria cervicornis* Gracilaria cervicornis was lower than some edible red seaweeds; e.g. *Gracilaria cervicornis* (22.96%), *Hypnea japonica* (19.00%) and *Porphyra tenera* (34.20%) [6,25,26]. However, this result was higher than the mean percentage of protein of other edible green seaweeds. According to Norziah and Ching [27], the mean percentage of protein content (19.72±0.77%) recorded from *C. racemosa* was higher than the concentrations found in higher plants. In addition, the protein content of *C. racemosa* was within the range of 10-47% for green seaweeds reported by [3]. Compare to those reported in other edible seaweeds, the mean percentages of crude lipid and fibre content of *C. racemosa* was much higher. The higher content of protein, crude lipid and fibre in *C. racemosa* species, probably due to the suitable environmental conditions such as temperature, salinity and nutrients of St. Martin Island.

Typically, seaweeds are not considered to be good source of lipid content, while most seaweed contains less than 4% of crude lipid at dry weight basis [28]. On the other hand, *Dictyota acutiloba* (16.1% DW), *Sargassum vulgare* species (7.8% DW) and *D. sandvicenis* (20.2% DW) were found to having higher amount of lipid content [28,29]. In the present study, remarkable percentage of crude lipid (7.65±1.19%) was recorded in *C. racemosa*. This higher percentage of crude lipid probably due to the use of different lipid extraction methods [18] or geographical and seasonal factors [29]. A number of studies were suggested that ash content could be varied ranged between 8-40% at dry weight basis [1]. The mean percentage of ash contents (12.15±0.46%) of *C. racemosa* was considerably lower than the other green seaweeds. In general, high level of ash was associated with the amount of mineral elements. The mean percentage of ash found was comparable to those reported in other species i.e., *Sargassum vulgare* (14.20%) and *Gelidium pristoides* (14.00%) [26,30]. The variation of ash content in seaweed usually depends on species, geographical distribution and their method of mineralization [31,32].

Comparison of the present results with previous studies for nutrient contents and amino acid compositions is difficult due to the lack of previous studies on nutritional composition of macro algae from this sub-tropical coast. The study revealed that glutamic acids and aspartic acids was the most abundant amino acid in *C. racemosa*. A number of study argued that green seaweed tended to show lower percentages of both aspartic acid and glutamic acid [6,33]. The mean percentage of aspartic and glutamic acid of *C. racemosa* (9.2±0.7% and 12.7±0.2%) obtained from this study was similar with *Codium taylorii* (10.1±0.2% and 10.7±0.1%), *Codium decorticatum* (10.8±0.3% and 12.0±1.1%) and *Codium taylorii* (10.6±0.4% and 11.3±0.4%; respectively) [33]. A good number of seaweeds have relatively higher content of free amino acids [16]. These amino acids added different flavours to several edible seaweeds. Glutamic acid gives a unique flavour and glycine and alanine give a sweet flavour to edible seaweeds [34]. Mean values for some essential (histidine, lysine, methionine and phenylalanine) and non-essential (glycine and serine) amino acids of *C. racemosa* was tended to be similar with other edible green seaweed (Table 3). However, compare to other edible green seaweed, *C. racemosa* was contained higher percentages of isoleucine (5.8±0.3% of total amino acid) and threonine (6.2±0.5% of total amino acids) and lower percentages of leucine (6.9±0.6% of total amino acids) and valine (5.1±0.3% of total amino acids).

In the context of developed country, people are more concern about risk and health issues [20]. On the other hand, with the increasing of education level, people from developing countries are now more aware about nutritional value of consumable food items [35]. Since *C. racemosa* is nutritionally rich, available and very cheap in south Asian countries, therefore, it could be considered as a low cost food staff for human consumption. It is well argued that consumption of different edible seaweeds have a positive effect on human health because they can reduce blood lipid level, obesity and risk of coronary heart diseases [7,36-39].

Conclusions

With respect to the higher level of protein and balanced amino acid profile, *C. racemosa* has appeared to be an interesting potential source of plant proteins. The higher level of protein, crude lipid and fibre content of this green seaweed species has a great food value from the nutritional and biochemical point of view. The results obtained from this study suggested that *C. racemosa* could be utilized as a functional food ingredient in our food industry. Further studies on *C. racemosa* should be concerned about fatty acid composition, vitamins, non-starch polysaccharide constituents, and trace elements and sensory perceptions to provide more information for safer and more versatile utilization of this seaweed species.

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