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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

PROXIMATE CHEMICAL COMPOSITION AND AMINO ACID PROFILE OF TWO RED SEAWEEDS (*Hypnea pannosa* AND *Hypnea musciformis*) COLLECTED FROM ST. MARTIN'S ISLAND, BANGLADESH

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Abstract: The proximate composition and amino acid profile of two subtropical red seaweeds (*Hypnea pannosa* and *Hypnea musciformis*) were investigated. The total protein (16.31 ±0.72% DW for H. *pannosa* and 18.64±1.12% for *H. musciformis*) and dietary fibre (40.59±0.95% DW for *H. pannosa* and 37.92±1.44% for *H. musciformis*) were the two most abundant components in these seaweeds. The mean percentage (dry weight basis) of crude lipid, carbohydrates and ash were 1.56 ±0.22%, 22.89 ±1.26% and 18.65 ±0.54% for *H. pannosa* and 1.27 ±0.41%, 20.60±1.68% and 21.57 ±1.04% for *H. musciformis*; respectively. *H. pannosa* and *H. musciformis* contained all the essential amino acids, which accounted for 52.27% and 53.89% of the total amino acids; respectively. Lysine, methionine and tyrosine were the limiting amino acids for *H. pannosa* (53.3, 14.6 and 25.8 mg/g protein; respectively) and *H. musciformis* (45.6, 16.2 and 26.2 mg/g protein; respectively). The proximate chemical composition and amino acid profile of these two red seaweed species exhibited high nutritional value for human consumption.

Keywords: *H. pannosa*, *H. musciformis*; Red seaweed, Proximate chemical composition, St. Martin's Island, Bangladesh

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Özet:

Bangladeş, St.Martin Ada'sından Toplanan İki kırmızı Deniz Alginin (*Hypnea pannosa* ve *Hypnea musciformis*) Amino Asit ve Kimyasal Besin Kompozisyon Profili

İki tane subtropikal kırmızı deniz alginin (*Hypnea pannosa* ve *Hypnea musciformis*) amino asit ve besin kompozisyonu incelenmiştir. Toplam protein (*H. pannosa* için kuru ağırlık üstünden % 16.31 ±0.72 ve *H. musciformis için* %18.64 ±1.12) ve besinsel lif (H. *pannosa için kuru ağırlık üstünden* %40.59±0.95 ve *H. musciformis* %37.92 ±1.44) değerleri ile bu alglerde en bol miktarda bulunan bileşiklerdir. Kuru ağırlık üzerinden yağ, karbonhidrat ve külün ortalama yüzde değerleri *H. pannosa için sırayla* %1.56 ±0.22, %22.89 ±1.26 ve %18.65 ±0.5 olup, *H. musciformis* için sırayla %1.27 ±0.41,%20.60 ±1.68 ve %21.57 ±1.04 dir. *H. pannosa* ve *H. musciformis* bütün esansiyel aminoasitleri içermekte olup sırayla toplam aminoasit içerikleri % 52.27 ve %53.89 olarak hesaplanmıştır. Lizin, methiyonin ve tirozin aminoasitleri *H. pannosa için sırayla* (53.3, 14.6 ve 25.8 mg/g protein) iken, *H. musciformis* için sırayla (45.6, 16.2 ve 26.2 mg/g protein) dir. Bu iki kırmızı alg türünün amino asit bileşimi ve besin değeri kompozisyonunun insan tüketimi açısından oldukça önemli düzeyde olduğu belirlenmiştir.

Anahtar kelimeler: *H. pannosa, H. Musciformis*, Kırmızı alg, Besin kompozisyonu, St. Martin adası, Bangladeş

Introduction

Utilization of seaweeds as plant protein source is of growing interest to the food industry in the developing countries (Fleurence, 1999; Wong and Cheung, 2000). People from Far East and Southeast Asian countries have a long tradition of consuming different types of seaweeds as a functional food (Mabeau and Fleurence, 1993; Darcy-Vrillon, 1993; Wong and Cheung, 2000). The applications of seaweed are not limited to human diet. Amongst 250 commercially utilized seaweeds, more than 150 seaweed species are consumed directly by humans as fresh or dried form and rest of the species are used as animal feed, fertilizer, fungicide, herbicides and as thickening and gelling agents for various industrial applications including foods (Fleurence, 1999; Kumari *et al.*, 2010; Mabeau and Fleurence, 1993; Rupérez, 2002; Wong and Cheung, 2000).

In Bangladesh, a total of 200 marine algal taxa (seaweeds) have been reported from the Saint Martin Island, Chittagong coast and Cox's Bazar coast, Bangladesh, of which 98 taxa under 53 genera are reds (Aziz and Islam, 2009; Islam *et al.*, 2010). *Hypnea pannosa* and *Hypnea musciformis* are two commercially important red seaweeds in Bangladesh. About more than 100 households are engaged in seaweed collection by hand or push net from St. Martin's Island. Collected seaweeds are sun dried on shore and dried seaweeds are exported to Singapore, Myanmar and China. A very few local people of St. Martin' Island consume seaweeds in their diet, but other people of Bangladesh does not consume seaweed because they don't have enough knowledge about the nutritional value of seaweeds.

A number of studies showed that seaweeds are valuable sources of protein, fibre, fatty acids, vitamins, macro and trace elements, as well as important bioactive compounds (Darcy-Vrillon, 1993; Fleurence, 1999; Mabeau and Fleurence, 1993; Novaczek, 2001; Ortiz *et al.*, 2006). Some trace elements are rare in terrestrial plants but they are rich in seaweeds (Ito and Hori, 1989; Rupérez, 2002). Seaweed contains bioactive products that exhibit antibacterial, antiviral and antifungal properties (Trono, 1999). Some compounds of seaweeds control high blood pressure, level of cholesterol, and prevent strokes. These can also be used as remedy for rheumatism, diarrhoea, and for controlling the growth of tumour (Novaczek, 2001). It is also well argued that consumption of edible seaweeds have a positive effect on human health because they can reduce blood lipid level, obesity and risk of coronary heart diseases (Benjama and Masniyom, 2011; Zafar, 2005).

The nutritional properties of edible seaweeds are poorly studied in Bangladesh. The detailed nutritional compositions of *H. pannosa* and *H. musciformis* have not been determined in Bangladesh and nutritional data on these two species are not yet available. Therefore, the proximate chemical composition and amino acid composition of these two seaweed species has a great importance to make the species edible and commercially viable to the consumers. The aim of this study is to investigate the proximate chemical composition and amino acid composition of these two red seaweed species in order to evaluate their potential nutritive value.

Materials and Methods

Study location

The St. Martin's Island is situated in the extreme South-Eastern corner of Bangladesh (roughly between 20° 34′ - 20° 39′ N and 92° 18′ - 92° 21′ E) and separated by a channel of about 8 km from mainland. The average turbidity (Secchi disc) of in-shore waters of St. Martin Island ranges from 1.5 m to 8.0 m. Water temperature and salinity fluctuated from 22-29°C (Tomascik, 1997) and 21.0-33.5 PSU (Zafar, 2005), respectively, throughout the year. The Island has four coasts and naturally occurring seaweeds are not found in all these coasts. The western coast is suitable for proper development and growth of seaweeds. In this study, we have selected three sampling stations at a 200 m of distance in the Western part of the Island (Figure 1). *Hypnea* species were available throughout the year in these selected locations.

Seaweed collection and preservation

Hypnea pannosa and *Hypnea musciformis* have been collected randomly by hand-picking from the study area at the time of low-tide during the month of May 2005. Fresh samples were taken into plastic jars and then kept at 4-5 °C for few days. In the laboratory, samples were gently brushed under running seawater, rinsed with distilled water, dried with paper tissue and frozen at -20°C. Subsequently, the samples were freeze-dried in a Terroni Fauvel (model LH-1500) device. The dried material was powdered manually with the use of a mortar and pestle and kept in desiccators containing silica gel, at room temperature, until the chemical analysis.

Proximate composition analysis

The proximate compositions (Proteins, carbohydrate, crude lipid, fibre, ash and moisture contents) of seaweeds were determined according to the standard method (AOAC, 2000). Protein content was analysed by the Kjeldahl method with a conversion factor of 6.25 to convert total nitrogen into crude protein. Carbohydrate content was determined as the weight difference using protein, lipid, fibre, moisture and ash content data (James, 1996). Crude lipid was extracted from the seaweed powder in a Soxhlet extractor with petroleum ether (Siddique and Aktar, 2011; Siddique *et al.,* 2012). After ensuring complete extraction, petroleum ether was evaporated and the residue was dried to a constant weight at 105°C. Fibre was quantified on 2 g samples previously boiled with diluted H_2SO_4 (0.3 N). The mixture was filtered and washed with 200 ml of boiling water and NaOH (0.5 N). The residue was re-extracted, after washed with boiling distilled water and acetone and finally dried at 105˚C to constant weight. The material was heated at 550˚C for 3 h and the weight was recorded. The moisture content was determined by drying the seaweed samples in an oven at 105˚C until a constant weight was obtained. The ash content was obtained by calcinations in a muffle furnace at 550˚C for 4 h.

Amino acid analysis

Amino acid analysis was carried out by ion-exchange chromatography under the experimental conditions recommended for protein hydrolysates. Samples containing 5.0 mg of protein were acid hydrolysed with 1.0 ml of 6 N HCl in vacuum-sealed hydrolysis vials at 110°C for 22 h. Norleucine was added to the HCl as an internal standard. Tryptophan, cystine and cysteine are completely lost by acid hydrolysis. Hydrolysates were suitable for analysis of all other amino acids. The tubes were cooled after hydrolysis, opened, and placed in a desiccator containing NaOH pellets under vacuum until dry (5-6 days). The residue was then dissolved in a suitable volume of a sample dilution Na-S buffer, pH 2.2 (Beckman Instr., USA), filtered through a Millipore membrane (0.22 μm pore size) and analysed for amino acids by ion-exchange chromatography in a Beckman (model 7300) instrument, equipped with an automatic integrator. The ammonia content was included in the calculation of protein nitrogen retrieval, as it comes from the degradation of some amino acids during acid hydrolysis (Mossé, 1990; Yeoh and Truong, 1996).

Statistical analysis

Data were expressed in terms of mean ± standard deviation and the mean values were analysed by one-way ANOVA to detect significant differences between the species. Statistical analysis of this study was done by Statistical Package for Social Science (SPSS Version 16.0 for windows).

Results and Discussion

Proximate chemical composition

The results from the proximate chemical composition of *H. pannosa* and *H. musciformis* showed that both of the species are rich in protein and dietary fibre. The mean percentage (dry weight basis) of protein and dietary fibre were found 16.31% and 40.59% for *H. Pannosa* and 18.64% and 37.92% for *H. musciformis* (Table 1); which were significantly different between these two species. When compared with *H. pannosa*, *H. musciformis* contained higher percentage of crude protein and ash content. However, the mean percentage (dry weight basis) of crude lipid, carbohydrates and ash were 1.56%, 22.89% and 18.65% for *H. pannosa* and 1.27%, 20.60% and 21.57% for *H. musciformis*; respectively (Table 1).

The mean percentages of protein in *H. pannosa* (16.31% DW) and *H. musciformis* (18.64% DW) were within the range of 10-47% for red seaweeds suggested by Fleurence (1999). Consistent with the previous studies, the results of this study confirmed that red seaweed contains higher amount of protein and dietary fibre than the green and brown seaweeds (Arasaki and Arasaki, 1983; Ratana-arporn and Chirapart, 2006). The mean percentage of protein obtained from *H. pannosa* and *H. musciformis* were higher than *Gracilaria cornea* (5.47% DW), *Gracilaria changgi* (6.90% DW), *Eucheuma cottonii* (9.76% DW) and *Gelidium pristoides* (11.80% DW) (see Table 2).

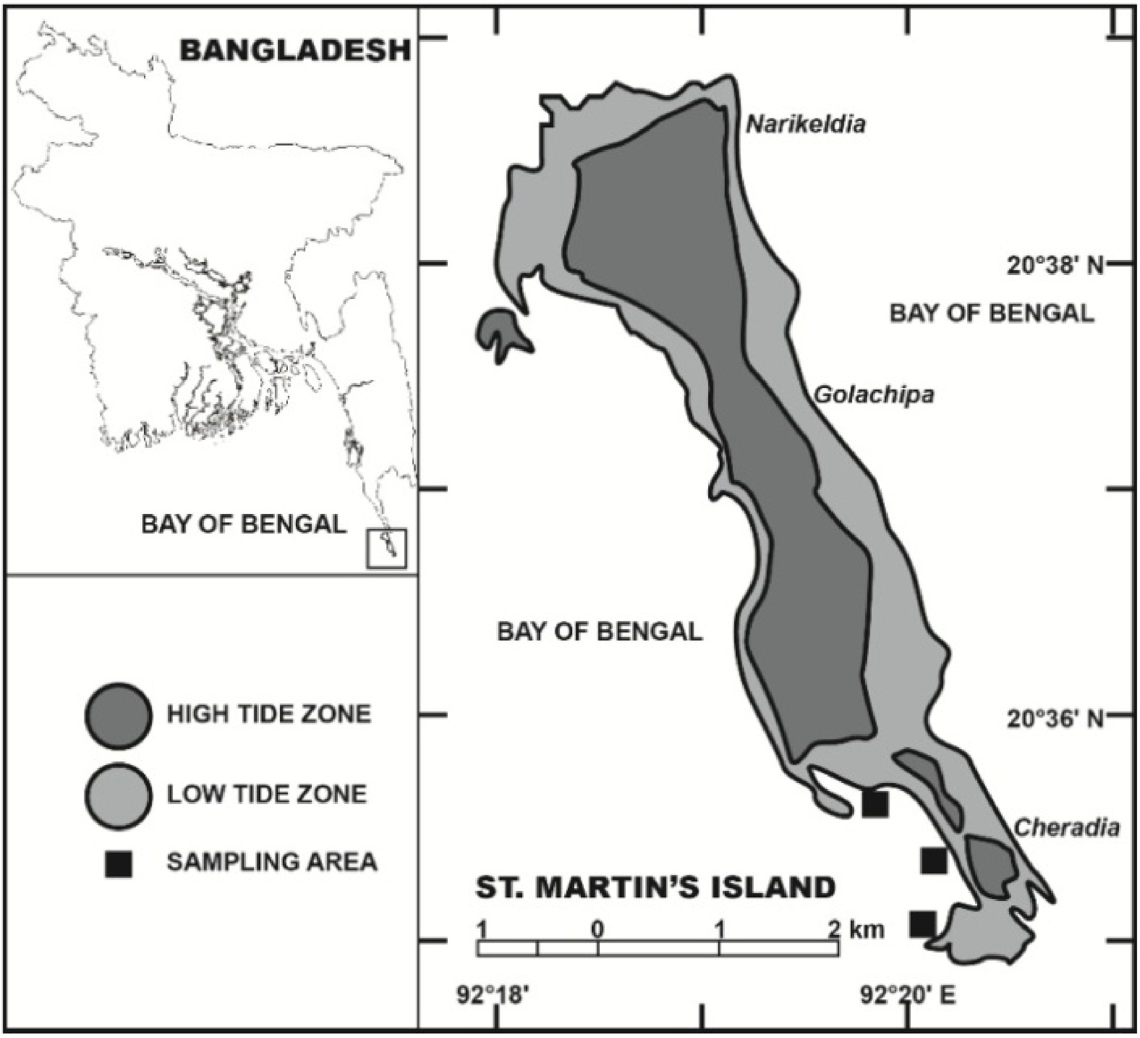

Figure 1. Showing the sampling locations at St. Martin's Island, Bangladesh

In general, seaweeds are not considered to be good source of lipid content (Norziah and Ching, 2000). According to McDermid and Stuercke (2003) most of the seaweed contains less than 4% crude lipid at dry weight basis. In the present study, the mean percentages of *H. pannosa* (1.56% DW) and *H. musciformis* (1.27% DW) were in recommended levels for lipid content. Although, the present results were higher compared to some other red seaweeds; *Gracilaria cervicornis* (0.43% DW), *Gelidium pristoides* (0.90% DW), *Porphyra tenera* (0.70% DW), but the similar percentage of lipid were found in *H. japonica* (1.42% DW) and *H. charoides* (1.48% DW) (Table 2).

The present study revealed that *H. pannosa* and *H. musciformis* are rich in dietary fibre. Although, the mean percentage of fibre in *H. pannosa* and *H. musciformis* were slightly lower than *H. japonica* (53.2% DW) and *H. charoides* (50.3% DW), but it was much higher than the other red seaweeds reported in the previous studies (see Table 2). The higher amount of dietary fibre in *H. pannosa* and *H. musciformis* were probably due to the suitable environmental conditions such as temperature, salinity, water transparency and higher input of nutrients at St. Martin Island (Haroon *et al.*, 2000). The mean percentage of carbohydrates in *H. pannosa* (22.89% DW) and *H. musciformis* (20.60% DW) were comparatively lower than other red seaweeds; but higher than *H. japonica* (4.28% DW) and *H. charoides* (7.02% DW) (Wong and Cheung, 2000).

The mean percentages of ash content found in *H. pannosa* (18.65% DW) and *H. musciformis* (21.57% DW) were similar with other red seaweeds (see Table 2). The mean percentages of ash in these two species were within the suggested levels of ash ranged between 8-40% at dry weight basis (Mabeau and Fleurence, 1993). Some previous studies were argued that the variation in ash content depends on seaweed species, geographical origins and their method of mineralization (Nisizawa, 1987; Sanchez-Machado, 2004). However, the mean percentages of ash found in this study were comparable to those reported in other species i.e., *Hypnea japonica* (22.10% DW), *Hypnea charoides* (22.80% DW), *Gracilaria changgi* (22.70% DW) (Norziah and Ching, 2000; Wong and Cheung, 2000).

Amino acid composition

The two red seaweed *H. pannosa* and *H. musciformis* contained all the essential amino acids, which accounted for 52.27% and 53.89% of the total amino acids; respectively. Lysine, methionine and tyrosine were the limiting amino acids for *H. pannosa* (53.3, 14.6 and 25.8 mg/g protein; respectively) and *H. musciformis* (45.6, 16.2 and 26.2 mg/g protein; respectively) (Table 3). The amount of Lysine in *H. pannosa* and *H. musciformis* were slightly lower compared to *Hypnea japonica* (66.3 mg/g protein), *Hypnea charoides* (64.9 mg/g protein) (Wong and Cheung, 2000). Furthermore, the level of other essential amino acids in *H. pannosa* and *H. musciformis* were above the FAO/WHO (1991) requirement pattern (see Table 3). With respect to the FAO/WHO (1991) requirement pattern, these two red seaweed species seemed to be able to contribute adequate levels of total EAA.

Table 1. Proximate chemical composition of *H. pannosa* and *H. musciformis* (dry weight basis, n = 3) collected from St. Martin's Island, Bangladesh.

Nutrient	*Hypnea pannosa*	*Hypnea musciformis*
Protein (%)	16.31 ± 0.72^{a}	18.64 ± 1.12^{b}
Crude lipid (%)	1.56 ± 0.22^{a}	1.27 ± 0.41^{a}
Carbohydrates (%)	22.89 ± 1.26^{b}	20.60 ± 1.68^{a}
Fibre (%)	40.59 ± 0.95^{b}	37.92 ± 1.44^{a}
Ash (%)	18.65 ± 0.54^{a}	21.57 ± 1.04^{b}
Moisture (%)	12.35 ± 1.41^{a}	11.54 ± 0.96^{a}

Note: Values are expressed as mean ± standard deviation, n=3; Values in the same row with different superscripts letters are significantly different ($p<0.05$).

Table 2. Proximate chemical analysis of different seaweed species reported by various authors

Species	Protein (%)	Lipid (%)	Carbohydrate (%)	Fibre (%)	Ash (%)	Moisture (%)	Reference
Eucheuma cottonii	9.76	1.10	26.49	5.91	46.19	10.55	Matanjun *et al.* (2008)
Gracilaria cervicornis	22.96	0.43	63.12	5.65	7.72	14.33	Marinho-Soriano *et al.* (2006)
Hypnea japonica	19.00	1.42	4.28	53.2	22.10	9.95	Wong and Cheung (2000)
Hypnea charoides	18.40	1.48	7.02	50.3	22.80	10.90	Wong and Cheung (2000)
Gracilaria changgi	6.90	3.30	-	24.7	22.70	-	Norziah and Ching (2000)
Gelidium pristoides	11.80	0.90	43.10	-	14.00	-	Foster and Hodgson (1998)
Gracilaria cornea	5.47	-	36.29	5.21	29.06	-	Robledo and Freile-Pelegrin (1997)
Porphyra tenera	34.20	0.70	40.70	4.80	8.70	-	Arasaki and Arasaki (1983)
Hypnea pannosa	16.31	1.56	22.89	40.59	18.65	12.35	Present study
Hypnea musciformis	18.64	1.27	20.60	37.92	21.57	11.54	Present study

Table 3. Amino acid composition of *H. pannosa* and *H. musciformis* and some other species of red seaweeds; values indicate the mean of three sampling stations with three replicates ± SD (n = 9).

Amino acids	*H. pannosa* (Present study)	*H. musciformis* (Present study)	*H. japonica*[d]	*H. charoides*[d]	FAO/WHO (1991) requirement pattern
Arginine[a]	51.9	68.3	66.8	63.6	
Histidine[a]	5.8	6.4	6.89	6.58	
Isoleucine[a]	48.2	42.1	44.8	48.5	28
Lysine[a]	55.3	45.6	66.3	64.9	58
Leucine[a]	77.4	84.8	97.9	72.3	66
Methionine[a]	14.6	16.2	18.5	16.8	25
Phenylalanine[a]	30.8	32.9	37.2	56.0	
Tyrosine[a]	25.8	26.2	27.9	26.0	63
Threonine[a]	41.5	62.7	45.9	51.3	34
Valine[a]	53.7	55.8	56.3	61.4	35
Alanine[b]	52.4	47.9	57.4	52.3	
Aspartic acid[b]	76.3	86.5	98.4	88.6	
Glutamic acid[b]	118.8	107.2	110	98.4	
Glycine[b]	49.2	47.7	54.2	50.6	
Proline[b]	40.8	44.2	45.4	47.9	
Serine[b]	32.3	43.9	47.5	44.9	
Total EAA	405 (52.27% of Total AA)[c]	441 (53.89% of Total AA)[c]	424	425	
Total amino acids (g/100 g DW)	14.8	15.3	17.3	16.2	

Note: [a] EAA (essential amino acids); [b] Non-EAA (non essential amino acids); [c] percentage of Total AA= [Level of total EAAs (mg/g of protein)/sum of all measured amino acids (mg/ g protein)] x 100; [d] Wong and Cheung (2000).

Serine and proline were found as limiting non-essential amino acids in *H. pannosa*; where, alanine and proline were the limiting amino acids in *H. musciformis*. The level of glutamic acid in *H. pannosa* (118.8 mg/g protein) was higher than the other *Hypnea* species. *H. musciformis* contained higher amount of aspartic acid (86.5 mg/g protein) than that of *H. pannosa* (76.3 mg/g protein). However, this amount was lower compared to other *Hypnea* species (*H. japonica* and *H. charoides*) (Wong and Cheung, 2000).

Comparison of the present results for amino acid composition with available data on seaweeds is difficult due to the lack of previous studies from Bay of Bengal (Zafar, 2005). A number of previous studies argued that red seaweeds has a tendency to show higher percentages of both aspartic and glutamic acids (Lourenço *et al.*, 2002; Wong and Cheung, 2000). In *H. pannosa* and *H. musciformis*, aspartic and glutamic acids constituted a substantial amount of the total amino acids (25.18% and 23.67% of total amino acid; respectively). Similar results were reported previously (Fleurence, 1999; Mabeau *et al.*, 1992; Wong and Cheung, 2000). The presence of aspartic and glutamic acids in food ingredient is very important since they provide different types of flavour and taste to several food items (Mabeau *et al.*, 1992; McLachlan, 1972).

Conclusion

The results from this study revealed that *H. pannosa* and *H. musciformis* are rich in protein and dietary fibre. The nutritional composition together with amino acid profile of the two red seaweed species suggested that they have potential food value and could be utilized as functional ingredient in our food industry. Further studies on *H. pannosa* and *H. musciformis* should aim fatty acid composition, vitamins, non-starch polysaccharide constituents, and trace elements and sensory perceptions to provide more detailed information for safer and more versatile utilization of these seaweeds.

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