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Nutritional composition and amino acid profile of a sub-tropical red seaweed *Gelidium pusillum* collected from St. Martin's Island, Bangladesh

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Abstract

Nutritional fact study has prime importance to make the species edible and commercially viable to the food consumers. The proximate chemical composition and amino acid profile of *Gelidium pusillum* were studied to understand the nutritional status. The red seaweed *Gelidium pusillum* was rich in dietary fibre (24.74 ± 1.05%), lipid (2.16 ± 0.61%) and ash content (21.15 ± 0.74%). The mean protein content (11.31 ± 1.02% DW) was within the range of 10-47% for green and red seaweeds and this range was higher than *Gracilaria cornea* (5.47% DW), *Gracilaria changgi* (6.90% DW) and *Eucheuma cottonii* (9.76% DW). *Gelidium pusillum* was found to contained all the essential amino acids, which accounted for 52.08% of the total amino acids. Tyrosine (26.2 mg g^{-1} protein), methionine (15.8 mg g^{-1} protein) and Lysine (48.3 mg g^{-1} protein) were the limiting amino acid of *Gelidium pusillum*. However, the levels of other essential amino acids were above the FAO/WHO requirement pattern (EAA score ranged from 1.14 to 1.62). Aspartic and glutamic acids constituted a substantial amount of the total amino acids (24.68% of total amino acid). The result from this study suggested that *Gelidium pusillum* could be utilized as a healthy food item for human consumption.

Introduction

For the last couple of decades, nutritionists and food scientists have given much more concentrations on nutritional evaluation of edible seaweeds (Ratana-arporn and Chirapart, 2006; Kumari *et al*., 2010). Most of the studies were focused on red seaweeds due to their higher nutritional value compared to other edible seaweeds (brown and green) (Arasaki and Arasaki, 1983; Wong and Cheung, 2000; Marinho-Soriano *et al*., 2006). Seaweeds have been using as a part of human diet in China, Japan, Thailand and South Korea for many years (Mabeau and Fleurence, 1993; Wong and Cheung, 2000). Depends on species, some seaweeds are generally suitable for making cool, gelatinous dishes or concoctions (Ito and Hori, 1989; Manivannan *et al*., 2009). In general, seaweeds are considered as low calorie food item, but rich in vitamin, mineral and dietary fibre (Ito and Hori, 1989). Seaweeds are also utilized as animal feed ingredient, row material for fertilizer and as well as various industrial applications (Mabeau and Fleurence, 1993; Fleurence, 1999; Rupérez, 2002; Kumari *et al*., 2010).

Approximately 1 million tonnes of wet seaweeds are annually harvested and extracted to produce hydrocolloids (McHugh, 2003). In addition, species belongs to *Gelidium* are among the most important agarophytes in the world (Santelices, 1974). About 35 seaweed species are harvested in various regions contributing to 40-50% of the world's annual exploitation of agarophytes (Whyte and Englar, 1981). Some seaweed is used in preparing creams, puddings, bears, wines, canned fishes etc. (Bold and Wynne, 1985). Several studies showed that seaweeds are valuable sources of dietary protein, lipid, fibre, vitamin and some essential minerals (Mabeau and Fleurence, 1993; Darcy-Vrillon, 1993; Fleurence, 1999; Novaczek, 2001; Ortiz *et al*., 2006). Although, it has always been realized that nutritional fact study has prime importance to make the species edible and commercially viable to the consumers (Wong and Cheung, 2000), the nutritional properties of edible red seaweeds are poorly studied in Bangladesh.

Gelidium, *Porphyra*, *Palmaria*, *Gracilaria*, and *Eucheuma* are the major edible red seaweeds (McLachlan, 1972), where, *Gelidium pusillum* is commonly known as "Lohit shoibal" in Bangladesh. This red seaweed species abundantly grows in the inshore water of St. Martin's Island, Cox's Bazar.

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Local people of St. Martin's Island usually do not consume this species, but they collect it from the intertidal water by push net or bamboo stick for their livelihood (Zafar, 2005). About 100 families of St. Martin's Island are engaged in collecting seaweeds. Collected seaweeds are sun dried on the sandy beach and export to Myanmar, Singapore and China (Siddique *et al.*, 2013). Previous study on proximate composition of *Gelidium pusillum* is very scantly and nutritional data on *Gelidium pusillum* is not available in the literature. However, proximate chemical composition and amino acid profile of *Gelidium pusillum* are examined in order to provide more comprehensive nutrient information about this species.

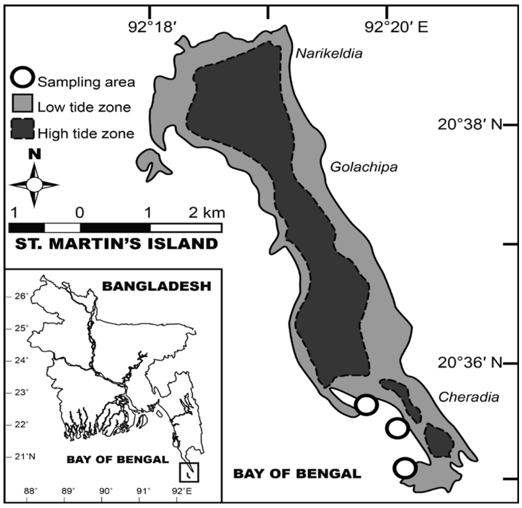

Figure 1. Study area and sampling stations in the St. Martin's Island, Bangladesh

Materials and Methods

Study area and sampling method

Gelidium pusillum has been collected randomly by hand-picking from the St. Martin's Island, Cox's Bazar at the time of low-tide during the month of April - June 2008. The St. Martin's Island is situated in the extreme South-Eastern corner (roughly between 20°34′ - 20°39′ N and 92°18′ - 92°21′ E) of Bangladesh which has naturally protected coral reefs. The average turbidity (Secchi disc) of in-shore waters of St. Martin's Island ranges from 1.5 m to 8.0 m. Water temperature of St. Martin's Island ranged between 22 and 29°C (Tomascik, 1997) and salinity of water fluctuated from 21.0-33.5 PSU throughout the year (Zafar, 2005). For this study three sampling stations has been selected at a distance of 200 m in the Western part of St. Martin's Island (see Figure 1). After collection, fresh samples were taken into plastic jar with ice and brought back to the laboratory immediately. In the laboratory, samples were washed by tape water for several times, then gently brushed and rinsed with distilled water. Finally, samples were dried with paper tissue and frozen at -20°C. The dried seaweeds were powdered manually using mortar and pestle and stored in desiccators until the chemical analysis.

Proximate composition analysis

The proximate chemical composition (protein, carbohydrate, crude lipid, fibre, ash and moisture content) of *Gelidium pusillum* was determined according to the standard method (AOAC, 2000). Protein content was analyzed by the Kjeldahl method. A conversion factor of 6.25 has been used to convert total nitrogen content into crude protein. Carbohydrate content was determined as the weight difference using crude protein, lipid, fibre, moisture and ash content data (James, 1996). Crude lipid of the seaweed was extracted from seaweed powder in a Soxhlet extractor with petroleum ether (Siddique and Aktar, 2011). After ensuring complete extraction of crude lipid, petroleum ether was evaporated and the residue was dried to a constant weight at 105°C. To determine the fibre content of seaweed 2 g samples were boiled with diluted H_2SO_4 (0.3 N). Then the mixture of sample and H_2SO_4 was filtered and washed with 200 ml boiling distilled water and NaOH (0.5 N). After washing by boiling distilled water and acetone the residue was re-extracted and dried at 105˚C to constant weight. The moisture content was determined by drying the seaweed samples in an oven at 120˚C until a constant weight was obtained and the ash content was estimated by heating the seaweeds in a muffle furnace at 550˚C for 4 h (Siddique *et al.*, 2013).

Amino acid analysis

Amino acid analysis was carried out by ion-exchange chromatography. 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 0.22μm Millipore membrane and prepared for amino acids analysis. A Beckman instrument (model 7300, USA) has been used for the ion-exchange chromatography. During the acid hydrolysis process, some ammonia content usually comes from the degradation of amino acids (Mosse, 1990; Yeoh and Truong, 1996). Therefore, the ammonia content was included in calculation of protein nitrogen retrieval. The contents of different amino acids recovered are presented as mg g-1 protein and are compared with the FAO/WHO (1991) reference pattern. The essential amino acid (EAA) score was calculated by the following equation:

Essential amino acid score = (mg of EAA in 1 g of test protein / mg of EAA in 1 g of egg protein) x 100

Statistical analysis

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

Results and Discussion

The proximate chemical composition of *Gelidium pusillum* are shown in Table 1. The mean protein content of *Gelidium pusillum* (11.31 ± 1.02% DW) was within the range of 10-47% for green and red seaweeds reported by Fleurence (1999). Several studies showed that red seaweed contains higher amount of protein and dietary fibre than that of some other green and brown algae (Arasaki and Arasaki, 1983; Ratana-arporn and Chirapart, 2006). Although, the mean percentage of protein obtained from *Gelidium pusillum* was lower than some edible red algae; e.g. *Gracilaria cervicornis* (22.96% DW), *Hypnea japonica* (19.00%), *Hypnea musciformis* (18.64% DW) and *Porphyra tenera* (34.20% DW), but it was higher than *Gracilaria cornea* (5.47% DW), *Gracilaria changgi* (6.90% DW) and *Eucheuma cottonii* (9.76% DW) (see Table 2). However, the mean percentage of protein content (11.31 ± 1.02% DW) recorded from *Gelidium pusillum* is higher than the concentrations found in higher plants (Norziah and Ching, 2000).

Compare to those reported in other edible seaweeds, the mean percentages of crude lipid (2.16 ± 0.61% DW) and fibre content (24.74 ± 1.05% DW) of *Gelidium pusillum* was slightly higher (Table 2). Edible seaweeds are not considered as a good source of lipid content as they contain less than 4% of crude lipid at dry weight basis (McDermid and Stuercke, 2003). The mean percentage of crude lipid (2.16 ± 0.61% DW) obtained from this study is higher than some edible red seaweeds (*Gracilaria cervicornis*, *Gelidium pristoides*, *Porphyra tenera*) reported in previous studies (Arasaki and Arasaki, 1983; Foster and Hodgson, 1998; Marinho-Soriano, 2006) (Table

Table 1. Proximate chemical composition of *Gelidium pusillum* (dry weight basis, n = 9) collected from St. Martin's Island, Bangladesh

Nutrient	*Gelidium pusillum*
Protein (%)	11.31±1.02
Crude lipid (%)	2.16±0.61
Carbohydrate (%)	40.64±2.21
Fibre (%)	24.74±1.05
Ash (%)	21.15±0.74
Moisture (%)	10.85±0.98

Note: Values are expressed as mean ± standard deviation, n = 9

2). Siddique *et al*. (2013) found 1.56% and 1.27% crude lipid in two subtropical red seaweed *Hypnea pannosa* and *Hypnea musciformis*, respectively from St. Martin's Island, Bangladesh. However, this result is still lower than the result obtained from *Gracilaria changgi* (3.30% DW) (Norziah and Ching, 2000). The mean percentage of fibre content (24.74 ± 1.05% DW) of *Gelidium pusillum* is much higher than other red seaweeds (Table 2), but this result is similar with the result found from *Gracilaria changgi* (24.70%DW) (Norziah and Ching, 2000). The higher amount of crude lipid and fibre in *Gelidium pusillum* were probably due to the suitable environmental conditions of St. Martin's Island (Haroon *et al.*, 2000). In *Gelidium pusillum*, the mean percentage of Carbohydrate was 40.64 ± 2.21% DW. This result concurred well with the previous report on *Gelidium pristoides* (43.10% DW) and *Porphyra tenera* (40.70% DW) (Arasaki and Arasaki, 1983; Foster and Hodgson, 1998).

The mean percentage of ash contents (21.15 ± 0.74%) found in *Gelidium pusillum* was similar with other red seaweeds. In general, high level of ash was associated with the amount of mineral elements. Previous studies reported that ash content of seaweed varies between 8 and 40% (at dry weight basis) (Mabeau and Fleurence, 1993). The mean percentage of ash found was comparable to those reported in other species i.e., *Hypnea japonica* (22.10% DW), *Hypnea charoides* (22.80% DW), *Hypnea musciformis* (21.57% DW), *Gracilaria changgi* (22.70% DW) (Norziah and Ching, 2000; Wong and Cheung, 2000; Siddique *et al*., 2013). Several other studies showed that the variation in ash content depends on seaweed species, geographical origins and their method of mineralization (Nisizawa, 1987; Sanchez-Machado, 2004).

Most of the essential amino acids found in *Gelidium pusillum*, which accounted for 52.08% of the total amino acid [Level of total EAAs (mg/g of protein)/sum of all measured amino acids (mg/ g protein) x 100%]. The amino acid profiles and the essential amino acid scores of *Gelidium pusillum* are presented in Table 3. Wong and Cheung (2000) observed that most of the essential amino acids in

Table 2. Proximate chemical composition of different red seaweed species reported by various authors (Values are given as percent of dry matter)

Species	Protein	Lipid	Carbohydrate	Fibre	Ash	Moisture	Reference
Gelidium pusillum	11.31	2.16	40.64	24.74	21.15	10.85	Present study
Porphyra tenera	34.20	0.70	40.70	4.80	8.70	-	Arasaki and Arasaki (1983)
Gelidium pristoides	11.80	0.90	43.10	-	14.00	-	Foster and Hodgson (1998)
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)
Gracilaria changgi	6.90	3.30	-	24.70	22.70	-	Norziah and Ching (2000)
Gracilaria cornea	5.47	-	36.29	5.21	29.06	-	Robledo and Freile-Pelegrin (1997)
Hypnea pannosa	16.31	1.56	22.89	40.59	18.65	12.35	Siddique *et al.* (2013)
Hypnea musciformis	18.64	1.27	20.60	37.92	21.57	11.54	Siddique *et al.* (2013)
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	50.30	22.80	10.90	Wong and Cheung (2000)

Table 3. Amino acid profile (mg g^{-1} protein) of *Gelidium pusillum*. Values are the average of three determinations and figures in parentheses are the essential amino acids score

Amino acids	*Gelidium pusillum* (Present study)	FAO/WHO (1991) requirement pattern
Arginine[a]	62.7	
Histidine[a]	4.6	
Isoleucine[a]	42.1 (1.50)	28
Lysine[a]	48.3 (0.83)	58
Leucine[a]	75.2 (1.14)	66
Methionine[a]	15.8 (1.62)	25
Phenylalanine[a]	31.9	
Tyrosine[a]	26.2 (0.97)	63
Threonine[a]	51.5 (1.51)	34
Valine[a]	44.7 (1.28)	35
Alanine[b]	51.6	
Aspartic acid[b]	82.2	
Glutamic acid[b]	108.8	
Glycine[b]	43.4	
Proline[b]	46.6	
Serine[b]	38.2	
Total EAA	403	
Total amino acids (g/100 g DW)	9.8	

Note: [a]EAA (essential amino acid); [b]Non-EAA (non essential amino acid)

some subtropical seaweed (*H. japonica*, *H. charoides* and *U. lactuca*), which accounted for 42.1-48.4% of the total amino acids. Furthermore, the levels of all their essential amino acids were comparable to those of the FAO/WHO (1991) requirement pattern. Tyrosine (26.2 mg g^{-1} protein), methionine (15.8 mg g^{-1} protein) and Lysine (48.3 mg g^{-1} protein) were the limiting amino acid of *Gelidium pusillum*. However, the levels of other essential amino acids in this study were above the FAO/WHO (1991) requirement (EAA score ranged from 1.14 to 1.62) (See table 3). With respect to the FAO/WHO (1991) requirement pattern, *Gelidium pusillum* seemed to be able to contribute adequate levels of total EAA for human.

This study revealed that glutamic and aspartic acids are the most abundant amino acids in *Gelidium pusillum.* A number of studies argued that red seaweed contains higher percentages of both aspartic and glutamic acids (Wong and Cheung, 2000; Lourenço, 2002). In *Gelidium pusillum*, aspartic and glutamic acids constituted a substantial amount of the total amino acids (24.68% of total amino acids). Similar results were reported in various other studies previously (Mabeau *et al*., 1992; Fleurence, 1999; Wong and Cheung, 2000). In general, most of the seaweeds contain relatively higher amount of free amino acids (Ratana-arporn and Chirapart, 2006). These amino acids provide different types of flavours to several edible seaweeds. Glycine and alanine give a sweet flavour to edible seaweeds (McLachlan, 1972) and aspartic and glutamic acids were responsible for the special flavour and taste of seaweeds (Mabeau *et al*., 1992).

With the increasing level of education in the developing countries, people are now more concern about nutritional value of consumable food items (Siddique, 2012; Siddique *et al*., 2012). Since *Gelidium pusillum* have good nutritional value, available and very cheap in south Asian countries, therefore, it could be considered as a low cost healthy food item for human consumption.

Conclusion

With respect to the higher level of crude protein and balanced amino acid profile, *Gelidium pusillum* appeared to be an interesting potential source of plant proteins for human consumption. The higher level of protein, crude lipid and fibre content of this red seaweed species has a great food value from the nutritional and biochemical point of view. The result of this study suggested that *Gelidium pusillum* could be utilized as a healthy food item for human consumption.

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