Potential of Gracilaria tenuistipitata var. liui grown in Nuniachara, Cox’s Bazar, Bangladesh

A. Aziz¹,³, A. Hassan¹, S. K. Roy¹, M. Z. Haque², B. K. Saha², S. Ahmed², M. Rahman², L. C. Mohanta² and O. F. Mashuk³

¹Department of Botany, University of Dhaka, Dhaka 1000, Bangladesh
²Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Kudrat-E-Khoda Road, Dhaka 1205, Bangladesh
³Adaptive Trials on Seaweed Cultivation along Bangladesh Coast Project, On-Farm Research Division, Bangladesh Agricultural Research Institute, Cox’s Bazar, Bangladesh

Abstract

During selection of Gracilaria tenuistipitata var. liui (red seaweed) growing naturally at Nuniachara sand-flat south-east of Moheshkhali channel, Cox’s Bazar local fishermen informed that Rakhaime and Chakma tribes of Cox’s Bazar and Bandarban consume the seaweed. To ascertain the quality of the organism as “Seafood” analysis of its nutritional status was carried out using three replicates in many cases. Percentage composition of total crude protein, crude fiber, crude lipid, carbohydrates, ash and moisture were 25.55 ± 0.18, 5.65 ± 0.13, 0.16 ± 0.03, 45.93 ± 1.53, 10.61 ± 0.69 and 12.10 ± 0.25%, respectively. Mineral contents such as phosphorus, calcium, magnesium, iron and copper were 596.90 ± 10.4, 132.75 ± 3.4, 3.90 ± 1.2, 80.13 ± 2.45 and 3.99 ± 1.2mg/100g dry wt., respectively. Heavy metals such as Pb, As, Cr and Cd were 0.031, 0.01, 0.06 and 0.02 mg/kg dry wt. respectively, which are below tolerance level. The seaweed contained 9.03% nine essential amino acids and 7.52% five non-essential amino acids. The quantity of total energy, β-carotene and vitamin C were 294.56 kcal/100g, 11.54 ± 1.20 and 2.5 mg/100g, respectively. Presence of high crude protein, amino acid profiles, β-carotene, phosphorus and low crude lipid and heavy metals made Gracilaria tenuistipitata var. liui to be considered as a good human food supplement.

Keywords: Gracilaria tenuistipitata var. liui; Red Seaweed; Nutritional composition; Food supplement; Cox’s Bazar, Bangladesh

Introduction

Seaweeds of family Gracilariaceae (Rhodophytaeae) are economically very important for valuable sources such as agar-agar (Santos 1990; Kumar and Fotedar 2009; Sousa et al., 2008), protein, fiber, fatty acids, vitamins, macro and trace elements as well as important bioactive compounds (Darcy-Vrillon 1993; Mabeau and Fleurence 1993; Fleurence 1999; Ortiz et al., 2006). Consumption of edible seaweeds has a positive effect on human health reducing blood lipid levels, obesity, and risk of coronary heart diseases (Crichtley et al., 2006; Benjama and Masniyom, 2011). In the Philippines Gracilaria tenuistipitata var. liui is consumed as a sea vegetable, and intensively cultivated in China and Taiwan for food (Haglund and Pedersen, 1993; Tseng and Xia, 1999). In Malaysia, several species e.g. G. changii and G. tenuistipitata are used as salads and for agar extraction (Phang, 2006). Agar is extensively used in the production of Jam and jelly, cosmetics, etc. and pharmaceutical industries, as well as in microbiological researches.

*Corresponding author e-mail: dr.aziz.botany@gmail.com
Due to the combined impacts of climate change, current cropping and consumption patterns, the seemingly plentiful and cheap resources are becoming limited and has been estimated that an equivalent of more than two Earths will be needed by 2050 to support the rapidly growing global population (Tiwari and Troy, 2015). Bangladesh is affected by population boom and frequent tidal bore along its 710 km coast against Bay of Bengal. Once crop fields are flooded with saline water about 7 years needed to produce land crops. Under the circumstances Bangladesh Agricultural Research Institute coordinated by BARC took a project on “Capacity building for conducting Adaptive Trials on Seaweed Cultivation in Coastal Areas” to provide nutritious seaweeds as food supplement (Anonymous, 2019; Aziz, 2015; Ito and Hori 1989). In fact the G. tenuistipitata var. liui growing on the sand-flat of Nuniachara beside Moshekhali Channel, Coxs’s Bazar is being consumed by Rakhaine and Chakma tribes. Therefore, nutritional analysis of the seaweed grown in large scale at Nuniachara has been carried out.

Materials and methods

Samples collection

Sundried sample of G. tenuistipitata var. liui (which on thorough study has been found to be a new record for Bangladesh (Aziz, 2020)) grown at Nuniachara sand-flat southeast of Moheshkhali Channel, Coxs’s Bazar under the Project “Adaptive Trials on Seaweed Cultivation in Coastal Areas”, implemented by Bangladesh Agricultural Research Institute, Gazipur, Coordinated by Bangladesh Agricultural Research Council, Farm Gate, Dhaka was supplied in zip-lock polybags for nutritional analysis.

Chemical analysis

Determination of crude protein

Crude protein content was determined by Micro-Kjeldahl method (Guebel et al., 1991). Powdered 0.5g sample was mixed in 10 ml of concentrated H2SO4 and 1:1 g digestion mixture (sodium sulphate and mercuric oxide) and heated for 6 h. It was diluted up to 100 ml with distilled water 10 ml of which was transferred in a micro-Kjeldahl distillation apparatus and distilled for 10 min. The distillate was collected in excess of 2% boric acid solutions with indicator and was titrated by 0.02 N HCl. It was compared with a similar blank digestion.

Determination of crude lipid

Crude lipid content of seaweed was determined by following Mehlenbacher (1960). Powdered 10 g sample was mixed with 150 ml n-Hexane and petroleum benzine solvents at 90:60 ratio. Crude lipid was extracted using Soxlet apparatus. Powdered 10 g extract was kept in the extraction thimble for eight hours and weighed again. Deference in the weight represented crude lipid content.

Determination of crude fiber

Crude fiber content was measured following AOAC (2000). In the moisture and fat free 5.0 g sample 200 ml of 0.255 N sulphuric acid was added and boiled for 30 min and after that 200 ml of 0.313 N (1.25%) NaOH solution was added to the solution and boiled for another 30 min. The filtrate was dried at room temperature and weighed. The sample was then kept in muffle furnace at 650 °C for 2-3 h, cooled and weighed again. The deference in weight represents the amount of crude fiber.

Determination of moisture

One gram-powdered sample at 25 °C was taken onto the tray of automatic moisture determination machine, Model Chyo, IB-30 for 10-15 min and moisture content was recorded.

Determination of ash

Ash content was determined by following AOAC (2000). The residue of crude lipid determination described earlier was considered as ash content.

Determination of carbohydrate

Total carbohydrate percentage was determined following Edeogu et al. (2007) using following formula- [Percentage of total carbohydrate content = 100 – (moisture + protein + lipid+ minerals)]

Determination of available energy

Available energy was calculated using the formula [available energy = (9.3 × fat) + (4.1 × carbohydrates) + (4.1 × protein)] described by Eneche (1991), Chinma and Igyor (2007) and Nwabueze (2007). The proportion of protein, fat, and carbohydrate was multiplied by their physiological fuel values of 4.1, 9.3, and 4.1 kcal, respectively, and the sum of the values was considered as available energy.

Determination of amino acids

Amino acid analysis was conducted by following the Amino Acid Analysis System Instruction Manual (Anonymous
1993). The amino acid analyzer uses the system of a column (Amino-Na) packed with a strongly acidic cation exchange resin for separation. Amino acids injected and separated using a binary gradient eluting method and detected by post-column derivation detection and fluorescence detector of each amino acid at high sensitivity and 120 kg/cm² pressure.

**Determination of vitamins**

β-carotene content was determined by column chromatography and quantification by visible spectroscopy as described in AOAC (1990). Vitamin C was measured by HPLC method (Lakshanasomyna 1998; Knelfel and Sommer, 1985).

**Determination of minerals**

The mineral contents (Ca, Mg, Fe, Cu, and P) were determined following the methods described in the Manual of Laboratory Techniques (Anonymous, 2006; Boltz, 1958; Rupérez, 2002).

**Determination of heavy metals**

Heavy metals were analyzed using Atomic Absorption Spectrometer (AAS). The quantities of chemical elements present 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).

**Table I. Nutrient contents (% dry wt.) of G. tenuistipitata var. liui, comparison with species reported by others and Spirulina platensis (used as natural vitamins)**

| Seaweed           | Crude protein | Crude lipid | Crude fiber | Moisture | Ash    | Carbohydrate | Available Energy (kcal/100g) | Reference                      |
|-------------------|---------------|-------------|-------------|----------|--------|--------------|------------------------------|--------------------------------|
| **G. tenuistipitata var. liui** | 25.55 ± 0.18   | 0.16 ± 0.03 | 5.65 ± 0.13 | 12.10 ± 0.25 | 10.61 ± 0.69 | 45.93 ± 1.53 | 294.56                      | Present research               |
| **G. cervicornis** | 19.70 ± 2.7    | 0.43 ± 0.06 | 5.69 ± 0.74 | 14.33 ± 1.78 | 10.51 ± 1.56 | 63.13 ± 3.5 | 356.84                      | Marinho et al. (2006)          |
| **G. changgi**    | 6.90 ± 0.10    | 3.30 ± 0.2  | 24.7 ± 0.7  |          | 22.70 ± 0.6 |          | -                           | Norziah and Ching (2000)       |
| **G. cornea**     | 5.47 ± 0.44    | -           | 5.21 ± 0.12 |          | 29.06 ± 0.50 | 36.29 ± 0.44 | 201.25                      | Robledo and Freile -Pelegrin (1997) |
| **Spirulina platensis** | 58.42 ± 1.07   | 12.25 ± 0.39 | 7.83 ± 0.26 | 8.83 ± 0.26 | 8.51 ± 0.42 | Nd            | Nd                          | Toyub et al. 2011              |

(n = 3, Mean ± SE)

**Results and discussion**

**Nutrient composition**

Utilization of seaweeds as plant protein, vitamins, etc. sources is becoming more popular in the food industry in developing countries (Fleurence, 1999; Wong and Cheung, 2000) and also in developed countries like USA where every super shop displays seaweed food item (Prof. Charles Yarish, Connecticut University, pers. Communication).

The crude protein content in G. tenuistipitata var. liui was 25.55 ± 0.18%, higher than G. cervicornis, G. changgi and G. cornea but significantly lower than Spirulina platensis (58.42 ± 1.07%), a cyanobacterium known as super food (Table 1). Crude fiber 5.65 ± 0.13% was similar to G. cervicornis and G. cornea and slightly lower than S. platensis (7.83 ± 0.26%) (Table 1). Crude lipid in the present seaweed was much lower 0.16 ± 0.03% than G. cervicornis and G. changgi also significantly lower than S. platensis (12.25 ± 0.39%) (Table 1). Ash content in G. tenuistipitata var. liui was 10.61 ± 0.69% similar to G. cervicornis, higher than S. platensis (8.51 ± 0.42%), but lower than G. changgi and G. cornea (Table 1). Moisture content in G. tenuistipitata var. liui was 12.10 ± 0.25% significantly lower than G. cervicornis and higher than S. platensis (8.83 ± 0.26%) (Table 1). However, moisture content usually depends on the drying process. Carbohydrate content in G. tenuistipitata var. liui was 45.93 ± 1.53%, which is significantly lower than G. cervicornis, but higher than G. cornea (Table 1). The energy
content of *G. tenuistipitata* var. *liui* was good enough (294.52 kcal/100g) and higher than *G. corneoa* but lower than other *G. cervicornis* (356.84 kcal/100g) (Table I).

### Amino acid profile

*G. tenuistipitata* var. *liui* contained higher amount of crude protein and thus amino acid profile was determined. As many as 15 amino acids were found of which eleven were essential amino acids (EAA), lysine percentage was highest and histidine was lowest, and four were non-EAA of which glutamic acid was highest. Total Amino acids were 16.55% of which total EAA was 9.03% (Table II). Total non-EAA was 7.52% of which glutamic acid was highest followed by aspartic acid (Table II). *G. tenuistipitata* var. *liui* had significantly higher total amino acids than *G. corticata* but much lower than *G. changgi* (Table II).

### Vitamins

Vitamin C was approximately 2.5 mg/100g in *G. tenuistipitata* var. *liui* lower than *G. lemaneiformis* (3.4 mg/100g) (Fig. 1). The lower value may be due to the fact that vitamin C content varies depending on heating time and drying processes (Igwemmar *et al.*, 2013; Santos and Silva, 2008). β-carotene content was found to be significantly higher (11.54 ± 1.20 mg/100g) than *G. changgi* (5.02 ± 0.40 mg/100g) and other species (*G. birdiae*, *G. caudate*, *G. domingiensis* and *G. ferox*) (Fig. 2).

### Minerals

Among the minerals P content (596.90 ± 10.4mg/100g) was similar to *S. platensis* (600.0 mg/100g), and was much higher than other species reported by others (Table III). Other minerals determined in *G. tenuistipitata* var. *liui* was compared with different species of *Gracilaria*: Ca was 132.75 ± 3.4 mg/100g significantly lower than *S. platensis* (700.0 mg/100g), much lower than other compared species such as *G. changgi* (651 ± 5.2 mg/100g), *G. gracilis* (429.11 mg/100g) and *G. lemaneiformis* (139.13mg/100g); Fe was (80.13 ± 2.45 mg/100g) significantly lower than *G. changgi* (95.6 ± 3.7 mg/100g) and *S. platensis* (150.0 mg/100g), but far more higher than *G. lemaneiformis* (26.09 mg/100g) and *G. gracilis* (15.20 mg/100g); Cu was (3.99 ± 1.2 mg/100g) significantly higher than *G. changgi* (0.8 ± 0.1 mg/100g), *G. lemaneiformis* (0.28 mg/100g), and *S. platensis* (1.20 mg/100g), (Table III).

### Table II. Amino acid (% dry wt.) profile of *G. tenuistipitata* var. *liui* and comparison with species reported by others

| Sl no. | Amino acids | *G. tenuistipitata* var. *liui* (Present Research) | *G. corticata* (Kumar and Kaladharan, 2007) | *G. changgi* (Norziah and Ching, 2000) |
|--------|-------------|-----------------------------------------------|----------------------------------------|--------------------------------------|
| 1.     | Arginine    | 1.62                                          | 0.89                                   | 3.55                                 |
| 2.     | Histidine   | 0.50                                          | 0.33                                   | 1.91                                 |
| 3.     | Isoleucine  | 0.84                                          | 0.63                                   | 2.94                                 |
| 4.     | Leucine     | 1.54                                          | 1.06                                   | 3.66                                 |
| 5.     | Lysine      | 1.66                                          | 0.86                                   | 1.66                                 |
| 6.     | Methionine  | 0.66                                          | 0.15                                   | 2.0                                  |
| 7.     | Threonine   | 0.69                                          | 1.23                                   | 3.98                                 |
| 8.     | Tyrosine    | 0.63                                          | 0.44                                   | 0.94                                 |
| 9.     | Valine      | 0.89                                          | 0.90                                   | 3.17                                 |
|        | Total EAA   | 9.03                                          | 6.49                                   | 23.81                                |
| 10.    | Alanine     | 1.42                                          | 0.81                                   | 6.33                                 |
| 11.    | Aspartate   | 1.64                                          | 1.99                                   | 4.01                                 |
| 12.    | Glutamate   | 2.63                                          | 1.85                                   | 6.36                                 |
| 13.    | Glycine     | 0.89                                          | 1.03                                   | 0.71                                 |
| 14.    | Serine      | 0.94                                          | 1.12                                   | Not determined                       |
|        | Total Non-EAA | 7.52                             | 6.8                                    | 17.41                                |
Introduction

G. changii

Determination of crude protein

Institute, Gazipur, Coordinated by Bangladesh Agricultural

seaweeds as food supplement (Anonymous, 2019; Aziz,

Cultivation in Coastal Areas” to provide nutritious

and was titrated by 0.02 N HCl. It was compared with a

apparatus and distilled for 10 min. The distillate was

var.

Amino Acid Analysis System Instruction Manual (Anonymous

Nwabueze (2007). The proportion of protein, fat, and

described by Eneche (1991), Chinma and Igyor (2007) and

Determination of available energy

Ash content was determined by following AOAC (2000).

The residue of crude lipid determination described earlier

was considered as ash content.

Determination of heavy metals

Heavy metals were analyzed using Atomic Absorption

Determination of mineral contents

The mineral contents (Ca, Mg, Fe, Cu, and P) were

as described in AOAC (1990). Vitamin C was measured by

β-carotene content was determined by column

HPLC method (Lakshanasomya 1998; Knelfel and Sommer,

as described in AOAC (1990). β-carotene content was

Vitamin C was approximately 2.5 mg/100g in

G. tenuistipitata

G. cornea

Table III. Minerals (mg/100g dry wt.) in G. tenuistipitata var. liui and comparison with species reported by others and Spirulina platensis (used as natural vitamins)

| Seaweeds               | Ca  | Mg  | Fe   | Cu  | P     | Reference                  |
|------------------------|-----|-----|------|-----|-------|----------------------------|
| G. tenuistipitata var. | 13.275 | 3.90 | 80.13 | 3.99 | 596.90 | Present research           |
| liui (present sample)  | ± 3.4  | ± 1.2 | ± 2.45 | ± 1.2 | ± 10.4 | Norziah and Ching, 2000    |
| G. changgi             | 651.1 | Nd  | 95.6 | 0.8  | Nd    | Rasyid et al., 2019        |
| G. birdiae             | ± 5.2 | Nd  | ± 3.7 | ± 0.1 |       |                            |
| G. caudate             | 429.11| Nd  | 15.20 | Nd   | 57.01 | Wen et al., 2006           |
| G. domingiensis        | 139.13| Nd  | 26.09 | 0.28 | 189.97|                            |
| G. lemaniformis        | 700.0 | 400.0 | 150.0 | 1.2  | 600.0 | Jung et al., 2019          |
| Spirulina platensis    |      |     |      |      |       |                            |
Heavy Metals

Heavy metals determined in *G. tenuistipitata* var. *liui* was compared with the Provisional Tolerable Daily Intake (PTDI) recommended by Joint FAO/WHO Expert Committee on Food Additives (JECFA), Food Safety Authority of Ireland (Anonymous, 2009), and *G. foliifere*: the determined amount of heavy metals in the present organism were, Pb 0.031 mg/kg (tolerable limit is 0.1-0.3 mg/kg) lower than *G. foliifere*; total As was 0.01 mg/kg (tolerable limit is 0.12 mg/day for a 60kg adult), lower than total As of *G. foliifere* (0.033 mg/kg); Cr content in present sample was 0.06 mg/kg (tolerable limit 0.1 mg/kg) and higher than *G. foliifere* (0.007 mg/kg); Cd was 0.02 mg/kg (tolerable limit 0.2 mg/kg) significantly lower than *G. foliifere* (0.055 mg/kg) (Table IV). All the four heavy metals determined for *G. tenuistipitata* var. *liui* were lower than the permissible limit and *G. foliifere* in most cases.

| Seaweeds                  | Pb   | As   | Cr   | Cd   | Reference       |
|--------------------------|------|------|------|------|-----------------|
| *G. tenuistipitata* var. *liui* (present sample) | 0.031 | 0.01 | 0.06 | 0.02 | Present research |
| *G. foliifere*           | 0.091 | 0.033 | 0.007 | 0.055 | Nabil et al. 2008 |

From the above analytical results and discussions, it can be concluded that the red seaweed can be used as enriched health seafood like *Spirulina platensis* in Bangladesh (Table I and III) (Toyub et al., 2011; Jung et al., 2019).

Conclusion

*G. tenuistipitata* var. *liui* (red seaweed) growing/grown at Nuniachara sand-flat, Moheshkhali Channel, Cox's Bazar is a rich sources of protein, 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 made it a nutritionally rich and healthy sea vegetable. In the wake of global warming, Bangladesh’s coastal areas being more prone to natural disasters that affect crop-field soil by increased salinity, selection of crops and cropping patterns should be changed and seaweed crop appears to be a suitable candidate, adapting to the changing coastal environment, ensuring food security.

Acknowledgment

Results presented are a part of MS thesis, during 2016-17 session, Department of Botany, University of Dhaka funded by the National Science and Technology Fellowship 2017, Govt. of the People’s Republic of Bangladesh. Seaweed samples were provided by Mr. Mostak Ahmed, Senior Scientific Officer, On Farm Research Division at Cox’s Bazar, Bangladesh Agricultural Research Institute, Gazipur, and in charge of the Seaweed Cultivation Project at Coxes Bazar titled “Adaptive Trails on Seaweed Cultivation in Coastal Areas”, coordinated by Bangladesh Agricultural Research Council, Farm Gate, Dhaka and technical support for nutritional analysis by different laboratories of Bangladesh Council of Scientific and Industrial Research are gratefully acknowledged.

Table IV. Heavy metals (mg/kg dry wt.) found in *G. tenuistipitata* var. *liui* and comparison with species reported by others

| Seaweeds                  | Pb   | As   | Cr   | Cd   |
|--------------------------|------|------|------|------|
| *G. tenuistipitata* var. *liui* (present sample) | 0.031 | 0.01 | 0.06 | 0.02 |
| *G. foliifere*           | 0.091 | 0.033 | 0.007 | 0.055 |

References

Anonymous (1993), Amino acid analysis system instruction manual, Shimadzu HPLC amino acid analysis system, Analytical Instruments Division, Kyoto, Japan, pp 63-65.

Anonymous (2006), Food and Nutrition Board, Recommended Dietary Allowances, 12th Ed., National Acad. Press, Washington DC. p 139.

Anonymous (2009), Mercury, Lead, Cadmium, Tin, and Arsenic in Food, Food Safety Authority of Ireland, Toxicology Factsheet Series, Issue no. 1, p 13.

Anonymous (2019), Capacity building for conducting Adaptive Trails on Seaweed Cultivation in Coastal Areas, Project completion report. Project ID No. CN/FRP: CET II-seaweed. BARI, Gazipur, p 54.

AOAC (1990), Official methods of food analysis, 15th Ed., Williams S. (Ed), pp 152-164, Association of Official Analytical Chemists, Washington DC.
AOAC (2000), Official methods of analysis, Association of Official Analytical Chemists, Washington DC. p 771.  
Aziz A (2015), Seaweed, the future revenue of Bangladesh’s Coastal water. Pre-proposal Training on Seaweed Cultivation (Mimeo), 12 December 2015. Bangladesh Agri. Res. Council, Farm Gate, Dhaka. pp 6-21.  
Aziz A (2020), New records of seaweeds from south-eastern coasts of Cox’s Bazar District, Bangladesh. Accepted for publication in Bangladesh J. Plant Taxon.  
Benjama O and Masniyom P (2011), Nutritional composition and physicochemical properties of two green seaweeds (Ulva pertusa and U. intestinalis) from the Pattani Bay in Southern Thailand, Songklanakarin J. Sci. Technol. 33(5): 575-583.  
Boltz DF (1958), The colorimetric determination of non-metals. herausgeg. von D. F. Boltz. Reihe: Chemical Analysis, Vol. VIII. Int. Sci. Pub. Inc. New York. p 286. DOI: 10.1002/ ange.19600720735.  
Chinma CE and Igoyar MA (2007), Micronutrients and anti-nutritional contents of selected tropical vegetable grown in South East Nigeria, Nigerian Food J. 25: 111-116. DOI: 10.4314/ nifoj.v25i1.33659.  
Critchley AT, Ohno M and Largo DB (2006), World Seaweed Resources. An authoritative reference system. Version: 1.0, Margraf Publishers Amsterdam: ETI Bioinformatics. p 540.  
Darcy-Vrillon B (1993), Nutritional aspects of the developing use of marine macro algae for the human food industry, Int. J. Food Sci. Nutri. 44 (Suppl.1): 23-35. Corpus ID: 88751638.  
Edeogu CO, Ezeonu FC, Okaka CE and Elom SO (2007), Proximate Compositions of Staple Food Crops in Ebonyi State, South Eastern Nigeria, Int. J. Biotechnol. Biochem. 1: 1-8. ISSN-2141-2162 ©2011  
Eneche EH (1991), Biscuit-making potential of millet/pigeon pea flour blends, Plant Foods Human Nutrition 54: 21-27. DOI: 10.1023/a: 1008031618117.  
Florence J (1999), Seaweed proteins: biochemical, nutritional aspects and potential uses, Trends in Food Sci. and Technol. 10: 25-28. DOI: 10.1016/S0924-2244(99)00015-1.  
Garcia R and Báez AP (2012), Atomic Absorption Spectrometry (AAS) In: Tech Europe. Ed. Farrukh MA, pp 7-13. DOI: 10.5772/25925.  
Guebel DV, Nudel BC and Giulietti AM (1991), A simple and rapid micro-Kjeldahl method for total nitrogen analysis, Biotecnol. Tech. 5(6): 427-430. DOI: 10.1012/ac60038a038.  
Haglund K and Pedersen M (1993), Outdoor pond cultivation of the marine red alga Gracilaria tenuistipitata var. liui Zhang et Xia (Gracilariariales, Rhodophyta) in brackish water in Sweden. Growth, nutrient uptake, cocultivation with rainbow trout and epiphyte control, J. App. Phyco. 5: 271–284. ISSN: 0921-8971.  
Igwemmar NC, Kolawole SA and Imran IA (2013), Effect of heating on vitamin C content of some selected vegetables, Int. J. Sci. Tech. Res. 2: 209-212. ISSN: 2277-8616.  
Ito K and Hori K (1989), Seaweed: Chemical composition and potential food uses, Food Rev. Int. 5: 101-144. DOI: 10.1080/87559128909540845.  
Jung F, Krüger-Genge A, Waldeck Pand K’upper JH (2019), Spirulina platensis, a super food?, J. Cellular Biotechnol. 5: 43-54. DOI: 10.3233/JCB-189012.  
Knefel W and Sommer R (1985), HPLC method zurbestimmung von vitamin C in milch, molke und molkegetranken, Z Lebensm Unters Forsch. 181: 107-110. ISSN: 0044-3026.  
Kumar V and Fotedar R (2009), Agar extraction process for Gracilaria cliftonii, Carbohydrate Polv. 78: 813-819. DOI: 10.1016/j.carbpol.2009.07.001.  
Kumar V and Kaladharan P (2007), Amino acids in the seaweeds as an alternate source of protein for animal feed, J. Mar. Biol. Ass. Ind. 49(1): 35-40.  
Lakshanasomya N (1998), Determination on Vitamin C in Some Kinds of Food by HPLC, Bull. Dept. Med. Sci. 40(3): 347-357.  
Mabeau S and Fluorence J (1993), Seaweed in food products: biochemical and nutritional aspects, Trends Food Sci. Technol. 4: 103-107. DOI: 10.1016/0924-2244(93)90091-N.  
Marinho-Soriano E, Fonseca PC, Carneiro MAA and Moreira WSC (2006), Seasonal variation in the
chemical composition of two tropical seaweeds, *Biore, Technol.* 97: 2402-2406. DOI: 10.1016/j.biotech.2005.10.014.

Mehlenbacher VC (1960), The analysis of fats and oil, The Garad Press Publishing Champaign, Illinois. ASIN: B00JCVA0E0.

Nabil A, Al-Shwafi and Rushdi AI (2008), Heavy metal concentrations in extruded green, brown and red seaweeds from coastal waters of Yemen, the Gulf of Aden, *Environ Geol.* 55: 653–660. DOI: 10.1007/s00254-010-1015-0.

Norziah HM and Ching CY (2000), Nutritional composition of edible seaweed *Gracilaria changii*, Elsevier. *Food and Chem.* 68: 69-76. DOI:10.1016/S0308-8146(99)00161-2.

Nwabueze TU (2007), Nitrogen solubility index and amino acid profile of extruded African breadfruit (*T. africana*) blends, *Nigerian Food J.* 25: 23-35. DOI: 10.4314/nifoj.v25i1.33651.

Ortiz J, Romero N and Robert P (2006), Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillea antarctica*, *Food Chem.* 99: 98–104. DOI: 10.1016/j.foodchem.2005.07.027.

Phang SM (2006), Seaweed resources in Malaysia: current status and future prospects. *Aquatic Ecosystem, J. Aqua. Eco. Health and Management* 9(2): 185-202. DOI: 10.1080/146349 806007 10576.

Rasyid A, Ardiansyah A and Pangestuti R (2019), Nutrient composition of dried seaweed *Gracilaria gracilis*, *Indonesian J. Marine Sci.* 24(1): 1-6. DOI: 10.14710/ik.ijms.24.1.1-6.

Robledo D and Freile-Pelegri G (1997), Chemical and mineral composition of six potentially edible seaweed species of *Yucatan*, *Botanica Marina* 40: 301-306.

Rupérez P (2002), Mineral content of edible marine seaweeds, *Food Chem.* 79: 2326. DOI: 10.1016/s0308-8146(02)00171-1.

Santos PH and Silva MA (2008), Retention of Vitamin C in Drying Processes of Fruits and Vegetables-A Review, *Drying Technology* 26: 1421-1437. DOI: 10.1080/07373930802458911.

Sousa MB, Maria-dos S, Pires K, Barroso de Alencar D, Holanda-Sampaio AH and Saker-Sampaio S (2008), α, β- carotene and α tocopherol in fresh seaweeds, *Food Sci. Technol.* 28(4): 3-4. DOI: 10.1590/S0101-20612008004000030.

Tiwari BK and Troy DJ (2015), Seaweed sustainability: Food and non-food applications (Cap-1), p 472. ISBN: 0124186971.

Toyub MA, Uddin MZ, Miah MI and Habib MAB (2011), Growth Performance and Nutritional Analysis of *Spirulina platensis* in Different Concentrations of Papaya Skin Powder Media, *Bangladesh J. Sci. Ind. Res.* 46(3): 333-338. DOI: 10.3329/bsjis.v46i3.9039.

Tseng CK and Xia BM (1999), On the *Gracilaria* in the Western Pacific and the Southeastern Asia region, *Botanica Marina* 42: 209-217.

Wen X, Peng C, Zhou H, Lin Z, Lin G, Chen S and Li P (2006), Nutritional composition and assessment of *Gracilaria lemaneiformis* Bory, *J. Integrative Plant Biol.* 48(9): 1047-1053. DOI: 10.1111/j.1744-7909.2006.00333.x.

Wong KH and Cheung CK (2000), Nutritional evaluation of some subtropical red and green seaweed. Part I: Proximate composition, amino acid profiles and some physicochemical properties, *Food Chem.* 71: 475-482. DOI: 10.1016/S0308-8146(00)00175-8.