PROTEOMICS OF THE RED ALGAE *Pyropia umbilicalis* (PYROPIA, RHODOPHYTA)

Jannatul Farhana

Roll no: 0219/18

Registration no: 779

Session: 2019 (July- December)

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

August 2022

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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DEDICATE TO MY BELOVED AND RESPECTED PARENTS AND TEACHERS

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Abbreviations

%	Percentage
&	And
LC-MS	Liquid Chromatography Mass Spectrometry
℃	Degree Celsius
µg	Microgram
µL	Microliter
mL	Milliliter
et al	Et alii/ et aliae/ et alia
L	liter
gm	Gram
kV	Kilovolt
mg	Milligram
mm	Millimeter
cm	Centimeter
nm	Nanometer
m/z	Mass-to-charge ratio
Da	Dalton
BDNF	Brain Derived Neurotrophic Factor
DTT	Dithiothreitol
ACE	Angiotensin Converting Enzyme
DPP-IV	Dipeptidyl Peptidase-IV

Abstract

There has been only a little expansion of proteomics' use in the study of algae. This study details the process of determining the proteome of the economically significant red seaweed *Pyropia umbilicalis*. For determination of the molecular properties of the collected red seaweed sample a partial DNA test was carried out, that was analyzed in NCBI BLAST for sequence homology. A protein extraction method with a combination of treatment using cellulase enzyme, sonication and ammonium sulphate extraction was employed to extract *Pyropia umbilicalis* proteins. Extracted protein was digested using trypsin in-solution digestion, and air drying was employed to reduce the volume of digested sample. LC-MS analysis was used to identify the functional proteins which are as follows R-phycoerythrin beta chain, R-phycoerythrin alpha chain, B-phycoerythrin beta chain, C-phycocyanin alpha chain, C-phycocyanin beta chain, Ribulose bisphosphate carboxylase large chain, Allophycocyanin beta chain, and Allophycocyanin alpha chain. Total yield of extracted protein was 33.494±0.709066 %. and concentration of total extracted soluble protein was 0.75±0.002% following the modified version of the Bradford assay. These findings emphasize the potential of employing proteomic techniques to investigate the *Pyropia umbilicalis* protein function.

Key index words: *Pyropia umbilicalis*; DNA test; NCBI BLAST; proteomics; red seaweed.

Chapter-1: Introduction

1.1 Background:

The red seaweed species known as lavers are most commonly used in the food processing industry or as a source of chemicals that are beneficial to one's health. They are classified under the Porphyra and Pyropia genera respectively. Lavers were traditionally considered to be staple foods in certain parts of Asia; but, as more people became aware of the positive effects that eating them had on their health and as more countries began exporting processed food goods, consumption of lavers increased all over the world (Stoyneva et al., 2015). It is anticipated that the development of seaweed aquaculture around the world as a source of pharmaceuticals and biomaterials (such as Alga Technologies and Cyanotech, amongst others) would contribute to the growth of the laver sector (Pereira, 2011). Commercial production of laver products (including, but not limited to, gim snack, which is flavored laver, mareun-gim, which is dried laver, okazu nori, which is laver for side dish, yakinori, which is barbecued laver, zicai tang, which is laver soup, etc.) is concentrated in northeastern Asia, with South Korea, China, and Japan producing 99.87% of total world production in 2017 (FAO, 2019). This is a reflection of the traditional use of laver in these countries as well as the regional environmental conditions that are conducive to aquaculture (Levine and Sahoo, 2010).

As a food source, laver can be consumed either raw or after being processed (for example, dried, roasted, or seasoned), or it can be used as a source of nutrients that are good for one's health. In northern Asia, laver is consumed mostly as a side dish, and as a result, it is often regarded as a foodstuff rather than a source of health functioning elements. This is due to the fact that laver is low in protein. Studies on other edible seaweeds, such as green or brown algae, on the other hand, concentrated mostly on their non-food roles as sources of nutraceuticals, food additives, and biomaterials. This is in contrast to the studies on kelp, which focused on its food role. The nutritional values and bioactive components of algal species that have been associated to considerable health advantages have been revealed, underlining the potential for growth of the laver sector as

both an edible seaweed and a source of beneficial compounds. Laver is a source of these two things (Madhusudan et al., 2011). Because of the high level of vitamin C found in porphyra (Nori), it is utilized as an antiascorbic in Chinese medicine (Chen, 1973). Even in modern times, the herb Ecklonia kurome is known throughout Asia for its ability to assist in the restoration of lying-in ladies (Park and Peterson, 1991).

Porphyra, more commonly known as "nori" in Japan, "laver" in the United Kingdom, the United States, and Canada, "purple laver" in Britain and Ireland, "karengo" in New Zealand, "kim" in Korea, and "zicai" in China, is most commonly used as a food ingredient in the Japanese delicacy "sushi," which consists of roasted blades, fish, rice, and other ingredients. The annual harvest of porphyra comes in as close to one million metric tons wet weight. It is estimated that Japan alone produces an annual average of 400,000 tons (wet weight) of Porphyra, which is then processed into around 10 billion nori sheets that have a market worth of more than US$1.5 billion (Levine and Sahoo, 2010). China produced the most nori sheets, 4,000,000,000 (Levine and Sahoo, 2010).

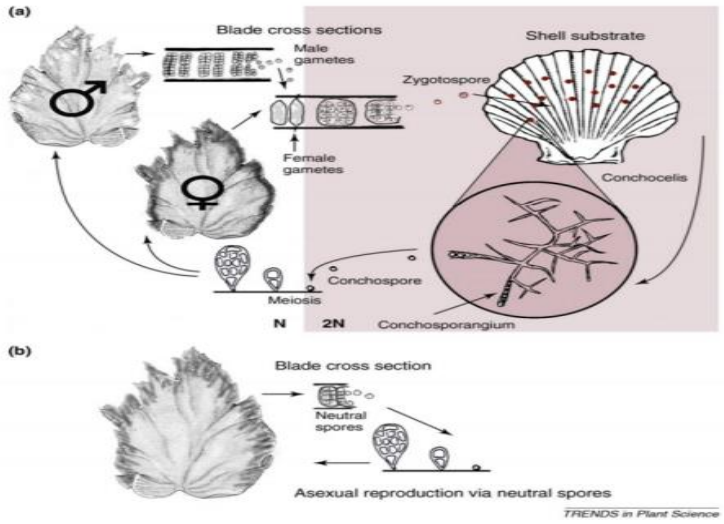


Figure 1.1: An overview of the life cycle of Porphyra umbilicalis (reproduced from Blouin et al., 2010).

Molecular methods allow us to tell *Porphyra umbilicalis* apart from other species in the genus, which is helpful because it is one of the most prevalent Porphyra species in the

Northwest Atlantic (Villalard-Bohnsack, 2003; Sutherland, 2011). *P. umbilicalis* in the northwestern Atlantic, on the other hand, does not have this alternation of generations and instead directly recycles blades from asexual neutral spores that develop on the blade borders. This differentiates its life cycle from those of other species of Porphyra and Pyropia (Blouin, 2010). *P. umbilicalis* blade thallus is made up of two parts: the holdfast and the vegetative regions, both of which have porphyran cell walls (Brawley et al., 2017). Different holdfasts and blades serve different purposes and have different physiological requirements. The photosynthesis, rapid cell division, and creation of neutral spores all take place in the blade, whereas the holdfast is responsible for attaching the organism to the rock (Royer et al., 2018).

Proteomics, the study of proteins, is the genomics of the protein world and has piqued the interest of biomolecular experts all over the world. It includes a wide variety of techniques used to study the three-dimensional structure and interaction partners of proteins, as well as their identification and the amount of protein expressed in cells. Proteomics is a fast expanding and critically essential area of study in the postgenomic age. Numerous higher plants, such as *Oryza sativa* L. (Komatsu et al. 2004), and Zea mays L., have benefited from the application of proteomic methods, which have helped to elucidate the roles of many proteins (Mechin et al. 2004). Transmembrane thykaloids and chloroplast protein expressions of the green algae Chlamydomonas reinhardtii are examples of subcellular compartment proteins studied by proteomics in algal research, cell wall of *Haematococcus pluvialis* Flotow (Wang et al., 2004).

Because of this, learning more about red seaweed through this study could shed light on their behavior; this knowledge would be crucial for boosting the commercial production and financial success of red sea-weed cultures. The field of in-silico analysis will benefit from this research in the future. Insights into the proteome profile of red seaweed could aid commercial food production of economically valuable species derived from this marine plant.

1.2 Objectives:

1. To extract protein from the red seaweed; *Pyropia umbilicalis*.
2. To analyze the extracted protein from *Pyropia umbilicalis.*
3. To identify the functional protein.

Chapter-2: Literature Review

2.1 Seaweed:

Macroalgae like seaweeds are algae. Though some species have a high protein content (up to 47%) and a complete necessary amino acid composition, the protein fraction is still poorly understood. Macroalgae have biological relevance due to their protein content and their ability to provide bioactive peptides and other proteinaceous substances. Enzymatic hydrolysis of seaweed to produce bioactive peptides has been the subject of numerous studies; examples include *Palmaria palmata* (Harnedy and FitzGerald, 2013; Beaulieu, et al., 2016), *Fucus spiralis* (Paiva et al., 2017), *Chlorella ellipsoidea* (Ko et al., 2012a), and *Porphyra columbina* (Cien et al., 2015). As a major producer in the food chain that releases oxygen into the ocean, seaweed plays a crucial role in maintaining marine ecosystems and is responsible for roughly 10 percent of the world's marine productivity. It's used in everything from food to hydrocolloid manufacturing to cosmetics and even fertilizer! (Chan et al., 2006).

Given their high protein, mineral, vitamin, dietary fiber, antioxidant, and fatty acid content and low caloric value, seaweeds are of great interest to those working in the field of nutrition (Khotimchenko et al., 2002; Agregán et al., 2017).

Even though its nutrient content is affected by external factors like habitat and environment, just like the majority of flora, seaweed is an excellent and natural source of nutrition (Gupta et al., 2011). It has many bioactive compounds that can be used as ingredients in functional foods and have many applications in human health. Despite the fact that its nutrient content is affected by such factors, seaweed is still a valuable source of nutrition. (Gupta et al., 2011).

Benefits to digestive health and prevention of chronic diseases like cardiovascular disease, diabetes, and cancer, as well as bacterial and viral disorders, have been linked to eating seaweed, according to some research (Rajapakse et al., 2011; Brown et al., 2014).

2.1.1 Applications of seaweed:

Since it can be used to make food, agar, and a gelling material, seaweed has great societal value. Seaweed is now being employed not just as a growth medium for bacteriological studies, but also in the food and pharmaceutical industries, which contribute significantly to its economic value (Pal et al., 2014). For over 14,000 years, particularly in Pacific and Asian countries, seaweed has been a staple of the human diet. Increased production and tolerance to biotic and abiotic difficulties are two of the many benefits of employing macroalgae in plant crops (Battacharyya et al., 2015). Applications of seaweed are listed in Table 2.1.1.

Table 2.1.1: Applications of seaweed.

Application	Reference
Food source such as bread, condiments, drinks, noodles, salad, soup, sushi	(Bouga and Combet, 2015)
Animal feed and supplement	(Schuenhoff et al., 2003)
For use in bacterial research, a suitable medium for growth	(Pal et al., 2014)
Restoration ecology	(Haapalehto et al., 2017)

2.1.2 Traditional Uses:

Certain thyroid conditions are typically treated with seaweeds in coastal areas. All of the Pen-suggested ts'ao's algae for goiter treatment (Laminaria digitata, Laminaria saccharina, Fucus vesiculosus, Sargassum). Traditional Chinese medicine's first record of using Sargassum seaweed ("Hai Zao") to cure goiter dates back to before 25-220 AD, when it was originally recorded in the "Shen Nong Ben Cao Jing" (Liu et al., 2012). In reality, this is among the most significant historical use of Sargassum seaweed. One of the most significant claims made for Sargassum in traditional Chinese medicine is that it can heal disorders related to the thyroid, such as goiter (Liu et al., 2012). In order to treat goiter, the South American Indians reportedly highly prized *Sargassum baccifer*

(Scwhimmer and Schwimmer, 1955). Historically, people in the Andean regions of South America have chewed "goiter sticks" to reduce the prevalence of thyroid enlargement (Schwimmer and Schwimmer, 1955). *Ecklonia maxima* (Osbeck) and other seaweeds ("seebamboes") are used as an iodine source in Cape Dutch medical botany.

2.1.3 Red seaweed:

There has been a lot of back and forth on how to classify the roughly 9600 species of seaweeds that have been described so far. Most commonly, seaweeds are divided into the three major types of red (Rhodophyta), green (Chlorophyta), and brown (Phaeophyta) algae based on their pigmentation. Although the brown algal thalli are larger, the red seaweeds have the most diversity at over 7,000 species, followed by the brown seaweeds at 2030 and the green seaweeds at 600 marine species (Baweja et al., 2016). When it comes to eukaryotic algae, red algae are likely one of the oldest groups. Red algal study has progressed and expanded considerably since the release of Biology of the Rhodophyta by Peter Dixon in 1973 and Biology of the Red Algae by Cole and Sheath in 1990.

The most common types of red algae are fibrous (Polysiphonia), pseudoparenchymatous (Ceramium), and parenchymatous (Porphyra, Halymenia). The Rhodophyta, from the Greek for "pink" (rhodo) and "plant" (phyta), are a group of algae that are both old and incredibly common (Usov et al., 1992). Their red pigments—carotenoids and phycobiliproteins—are responsible for their photosynthetic abilities and unique polysaccharide composition; they don't have starch in their chloroplasts but instead store energy in florid cytoplasmic starch.

The blue phycocyanin and phycoerythrin pigments are found in Rhodophytes as well as the red carotenoid. Rhodophytes' red and blue pigments let them take advantage of the blue-green light from the ocean's surface during photosynthesis, wherein they produce food for themselves (Pal et al., 2014).

2.1.3.1 ***Pyropia umbilicalis*:**

The red algae known as Pyropia species belong to the phylum Rhodophyta, class Bangiophyceae, order Bangiales, and family Bangiaceae. These algae have a discoid holdfast and a short stipe. Pyropia's growth zone can range from the shoreline to depths

of up to ten meters, depending on the transparency of the water and the substrate it's growing on (Qian et al. 2015). They are exceptionally resistant to heat stress, and certain species of the genus Pyropia are even able to turn off metabolic functions like photosynthesis that aren't absolutely necessary for keeping their homeostasis stable (Xu et al.,2014). It has been demonstrated that several other species are able to fight dehydration by increasing their lipid production (Qian et al., 2015). As a potential additive (emulsion model system), the red algae *Pyropia umbilicalis* is being evaluated for use in meat products with the goal of improving their functional properties and increasing their nutritional value (Cofrades et al., 2013).

2.1.3.2 Protein content of red algae:

Analyses of red algae revealed a staggeringly high concentration of protein-18.8 7 g/100 g, on average. *Porphyra umbilicalis* contained the most (40.0 g/100 g) (Gamero-Vega et al., 2020). About half of the algal species in the table had a protein level of more than 15 g/100 g, while another 40 percent had a protein content of more than 20 g/100 g; these are extremely high values, on par with those seen in meats and dried legumes (Gamero-Vega et al., 2020). Compared to other forms of algae, Porphyra algae have an exceptionally high protein concentration, averaging 29.3 g/100 g. Porphyra, a traditional meal in Asia (especially China, Japan, and Korea), is high in protein and free amino acids and has a pleasant flavor, according to the Food and Agriculture Organization (2009). On average, red algae have more protein than their green and brown algae. One study found that the protein content of nori algae (*P. umbilicalis*) was comparable to that of soybeans (Palasí et al., 2015).

2.2 Proteomics:

Proteomics is the research approach of proteins, including their composition and physiological function. Proteins are the most basic biomolecules or parts of cells in all organisms. In 1997, the phrase proteome entered the lexicon. It resembled the word genome very much. Mark Wilkins, in 1994, invented the term proteome, which is a mixture of "protein" and "genome." Specifically, a proteome is the whole catalog of proteins made by a live organism. Protein-protein interactions must be studied in order to obtain a complete understanding of the function of proteins in a biological system.

Analysis of biological protein-protein interactions is a primary focus of proteomics research.

Proteomics is a new area, having emerged just 10 years ago; its quick development is related, in large part, to the tremendous advances in mass spectrometry (MS) over the past few years. The sole function of a mass spectrometer is to perform this measurement. The mass in proteomics is used to determine the protein's identity, chemical alterations, and structure (Parker et al., 2010). Multiple methodologies for peptide and protein quantification by LC-MS have been developed and deployed to address a wide range of biological concerns, and LC-MS has emerged as a formidable molecular biology tool (Cutillas et al., 2010). Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is allowing for global protein analysis thanks to the development of new quantitative methodologies and sophisticated bioinformatics tools to deal with the analysis of the vast volumes of data generated in proteomics research (Cutillas et al., 2010).

Chapter-3: Materials and Methods

3.1 Study area and study period:

The research was carried out over the course of a period of six months, beginning in January 2022 and ending in June 2022. At Chattogram Veterinary and Animal Sciences University in Bangladesh, the experimental process was carried out in the laboratory of the Department of Applied Food Science and Nutrition and the Poultry Research and Training Center (PRTC).

3.2 Sample Collection:

The red seaweed sample *Pyropia umbilicalis* was collected from Cox'sbazar and Patenga sea beach. It was collected with a sharp blade at low tide and cleaned the sample by separating it from stones, oysters, snails etc.

3.3 Sample Identification:

After collecting the red sea-weed sample, partial DNA test was done to identify the molecular characteristics of the sample. That sample was analyzed at NCBI Blast for sequence homology.

3.4 Materials Required:

Table 3.4: List of materials required for proteomics of red algae *Pyropia umbilicalis*.

Apparatus	Machinery	Reagents	Bioinformatic tool
Centrifuge tube	Magnetic stirrer	Acetate buffer	Uniprot (https://www.uniprot.org/)
Dialysis tube	Centrifuge machine	NaOH buffer	
Beaker	Sonicator	Ammonium sulfate	
Weight balance	Freeze drier	HCL	
Hot water bath		Na_2CO_3	
Blender		Deionized water	

3.5 Methods:

3.5.1 Sample Preparation:

The collected seaweed samples were washed three times using clean sea water and kept it under the shade for half an hour. Placed the sample in a cork sheet and add some liquid nitrogen in it for quickly frozen and stored it at at -80°C temperature for 2 days. Freeze drying was done by keeping the sample in a small tube and then powdered it using a blender. Finally, the sample was preserved in an ice bag at the chiller. The overall process of sample preparation is shown in the form of flowchart (Figure 3.5.1).

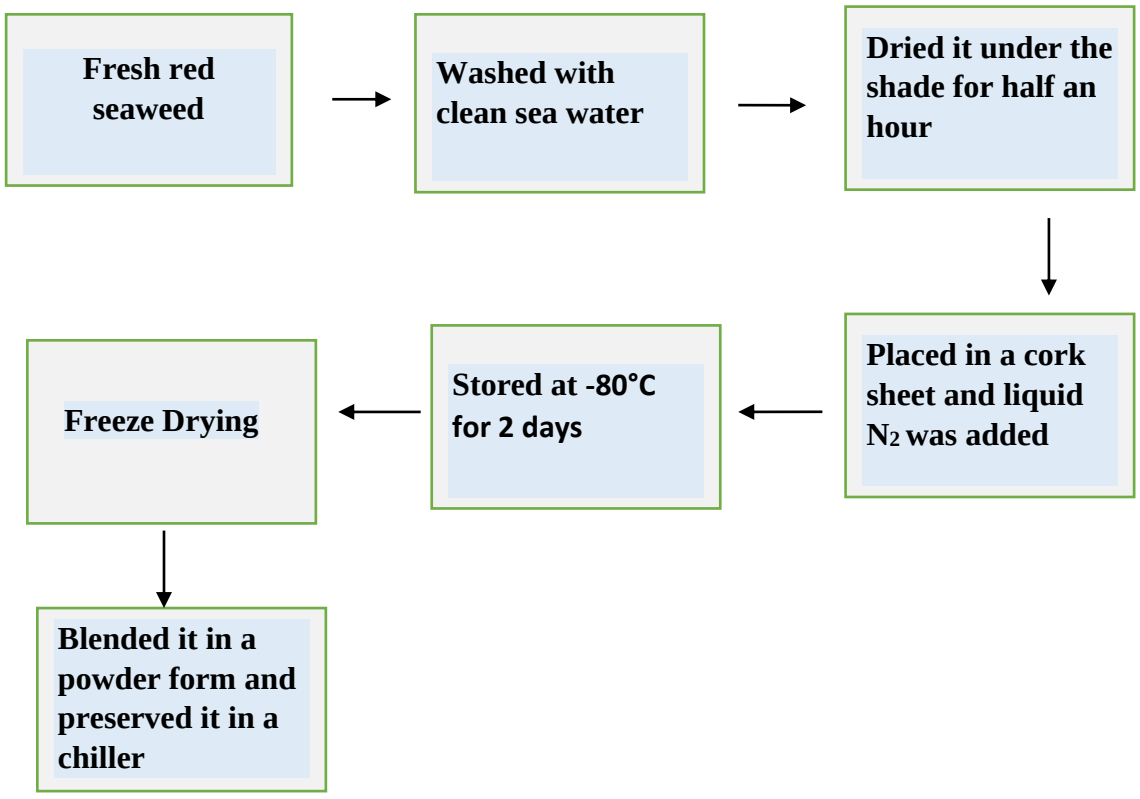


Figure 3.5.1: Flow chart of red seaweed sample preparation.

3.5.2 Protein extraction method:

Employing modified version of the technique described by Galland-Irmouli et al. (1999), I extracted proteins from seaweed. Cellulase enzyme treatment, sonication, and ammonium sulfate extraction were all part of the process. A powdered form of freeze-dried seaweed weighing 10 grams was suspended in a buffer containing 250 milliliters of

acetate and one gram of cellulase for two hours in a water bath (Shaker bath 903, Protech, UK). Then, 1 L of ultrapure water was added to the mixture, and it was sonicated for 1 hour. The seaweed solution was placed on a magnetic stirrer plate and left at 4°C to be swirled for a whole night. Decanting the supernatant after centrifuging the seaweed solution at 10,000 g for 30 minutes. The concentration of ammonium sulfate in the supernatant was increased to 60% (w/v). The protein fraction was precipitated by stirring the mixture at 4°C for 1 hour and then centrifuging it at 10,000g for 30 minutes. Overnight at 4°C, the precipitates were dialyzed against ultrapure water using 3.5-kDa MWCO dialysis tubing (Fischer Scientific, USA). The precipitate was then freeze-dried and kept at -80°C for further use. Protein extraction process is described in the form of flow chart (Figure 3.4.2).

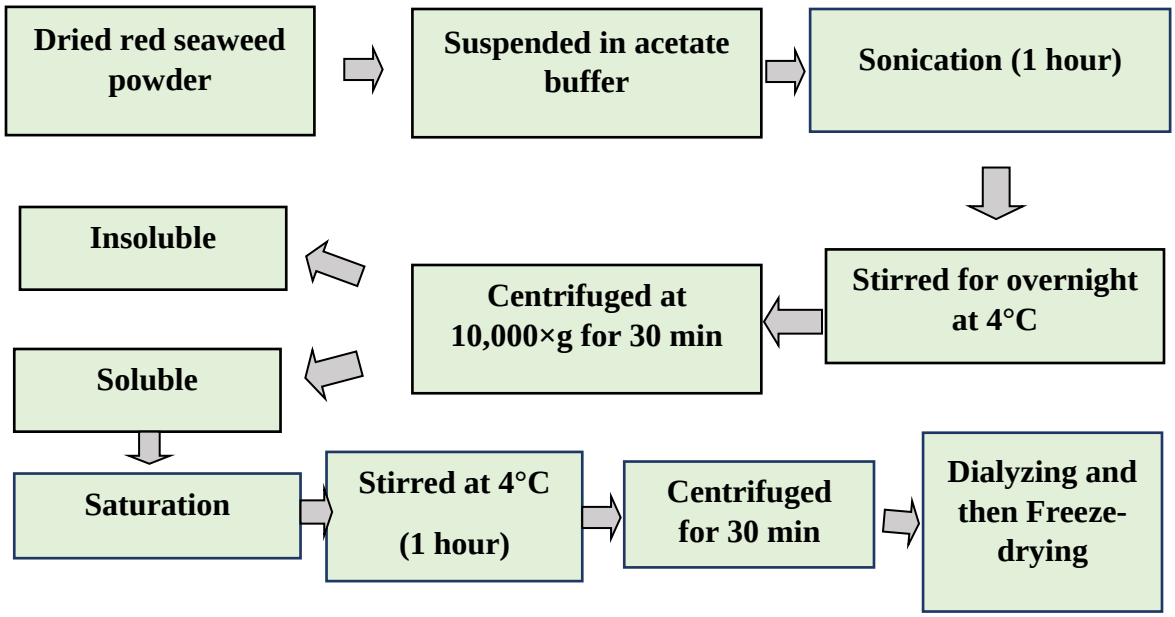


Figure 3.5.2: Flow chart of protein extraction from *Pyropia umbilicalis*.

3.5.3 Determination of yield of protein extract:

The yield of seaweed protein extract powder obtained from seaweed powder was calculated. The calculation was carried out in the following manner:

$$\text{Seaweed protein extract powder (\%)} = \frac{\text{Weight of seaweed protein extract powder}}{\text{Weight of freeze dried seaweed powder}} \times 100$$

3.5.4 Estimation of Total Soluble Protein Concentration:

The amount of total soluble protein found in the samples of seaweed was determined with the help of a modified version of the Bradford assay (Bradford, 1976), which was carried out on 96-well microtiter plates with reference to the Bio-Rad Protein Assay user manual. The bovine serum albumin standard protein curve was used to determine protein concentration (BSA). The Bradford reagent (BIORAD, Hercules, CA, USA) was diluted with distilled water five times before being used. Ten microliters of standard and protein extracts were combined with two hundred microliters of diluted Bradford reagent and left to remain at room temperature and out of the light for five minutes. The absorbance at 595 nm was measured using a Thermo Scientific microplate reader after 5 minutes of reaction on microtiter plates, as per the manufacturer's instructions (Multiskan Ascent V1.25 Plate Reader with Ascent Software version 2.6: Thermo Electron Corporation, Vantaa, Finland). Using the slope of a standard curve plotted in Excel, I was able to determine the protein concentration in the samples. Each sample's protein concentration was calculated using the calibration curve. Protein solubility is proportional to the 595 nm absorbance value, hence measuring this parameter provides a straightforward method for quantifying protein concentration. Calibration graph obtained from a set of BSA standards is shown in Figure 3.5.4. Since BSA is both cheap and easily accessible, it is frequently used as a protein standard. Because BSA is so commonly used as a protein standard, we can reliably evaluate our results against those of previous studies. There was a protein content of 0.01 µg/µL.

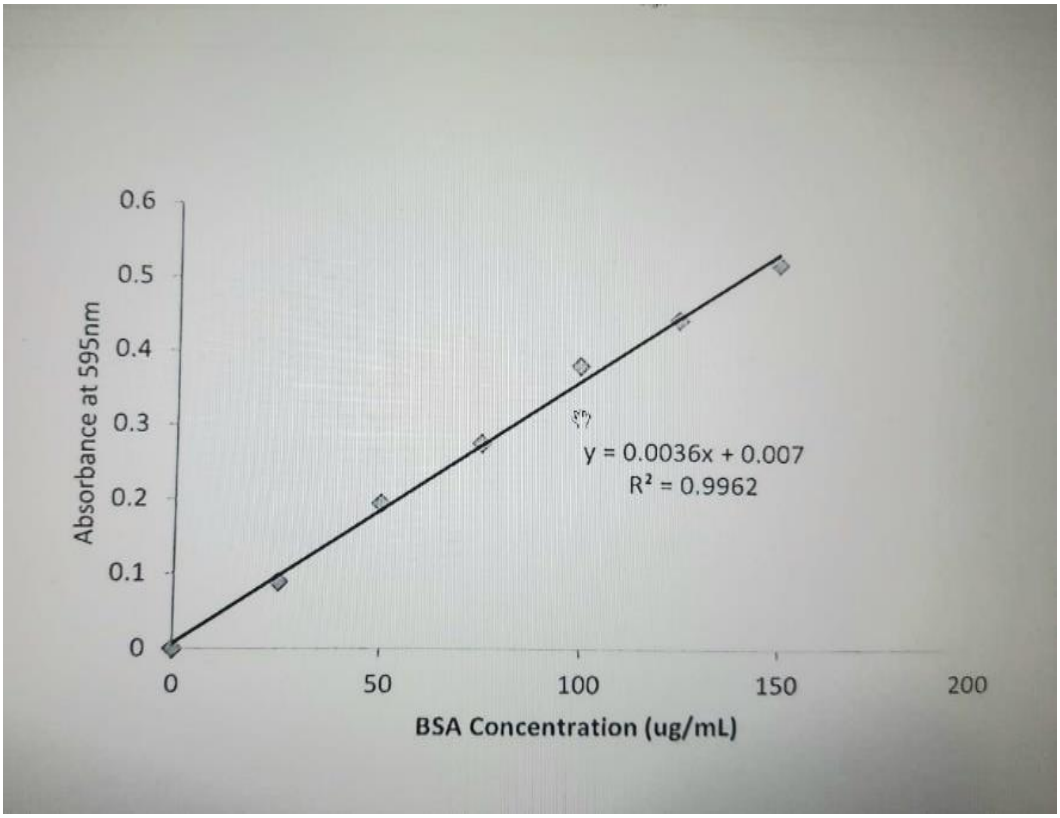


Figure 3.5.4: Calibration graph obtained from a set of BSA standards.

3.5.5 In-solution digestion:

After extraction, 1 mg of dried protein of red seaweed was digested using trypsin in-solution digestion method following Kinter and Sherman, with minor modifications. 100 L of a buffer composed of 6 M urea and 100 mM Tris-HCl buffer, pH 7.8, was used to re-solubilize the material. For the reduction step, 5 L of 200 mM DTT was added to each sample, and the resulting solutions were incubated at room temperature for one hour. Twenty µL of stock Alkylating agent, IAA (200 mM), was then added into the solutions to a final concentration of 10 mg/ml. and incubated for another hour at room temperature for the samples' alkylating process. The samples received a second addition of 20 µL DTT and were incubated for an additional hour Then, dilution was accomplished by adding 775 µl of deionized water. The samples were then treated with 20 µg of bovine trypsin dissolved in 100 µl of solution. The sample tubes were then placed in the

thermomixer and digested at 37 °C for eight hours. Concentrated acetic acid was added the following day to bring the pH of the fluid in the sample tubes down to below six, which ended the reaction. After the sample had been digested, it was air-dried in a vacuum concentrator until its volume was less than 20µ. In solution digestion procedure is shown in the form of flow chart (Figure 3.5.5).

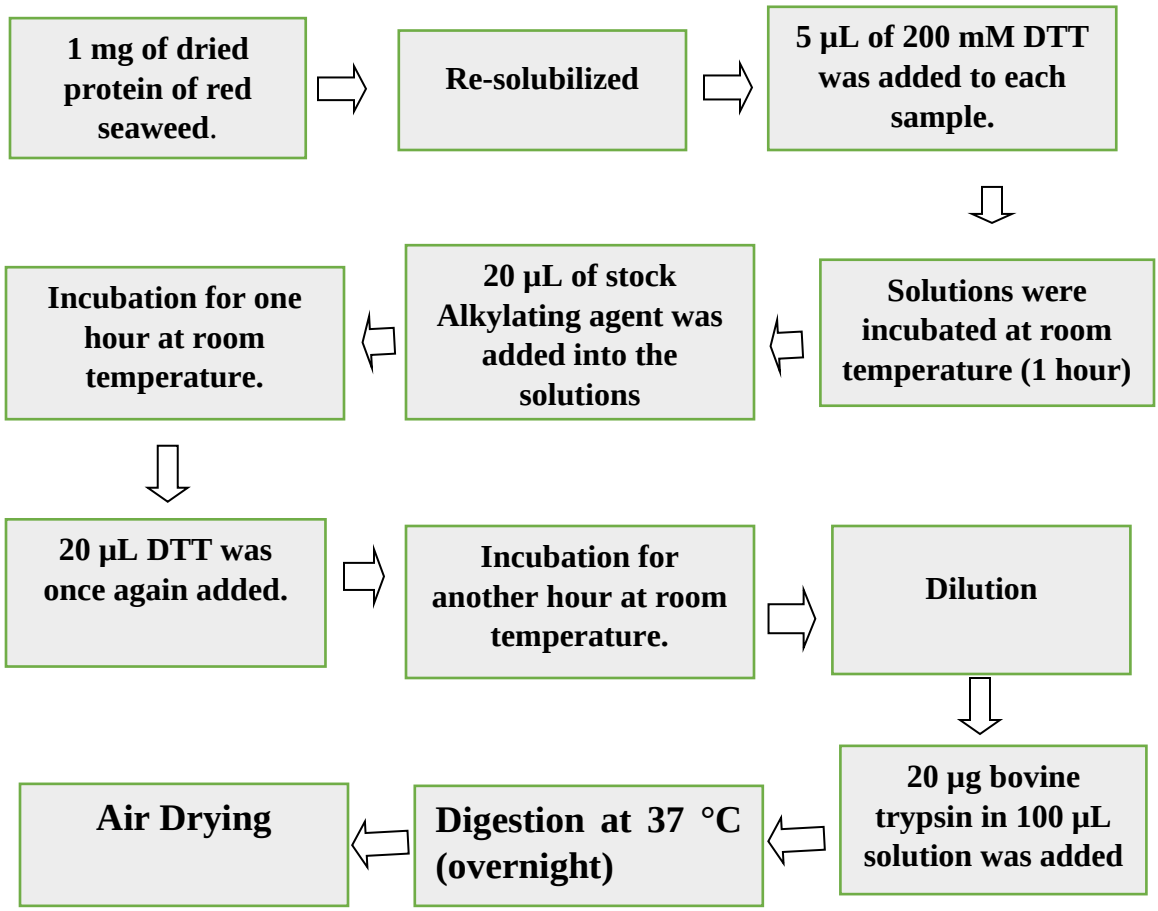


Figure 3.5.5: Flow chart of in-solution digestion

3.5.6 Mass spectrometry analysis:

The sample was diluted with 50 µL of 0.1% formic acid (FA) in deionized water before being analyzed by LC-MS/MS. After that, a regenerated cellulose (RC) membrane syringe filter with a 0.2 µm pore size (Sartorius AG, Goettingen, Germany) was used to filter the solution. Liquid Chromatography-Mass Spectrometry (LCMS) analyses were performed according to Kwan et al. (2015) with some modifications using LTQ-Orbitrap

Velos Pro mass spectrometer coupled to Proxeon Easy–nLC II nano-LC (Thermo Scientific, USA) with the running buffer of 0.1 % of FA in deionised water and 0.1 % of FA in 100 % acetonitrile (ACN). The columns utilised were Easy column C18 (10 cm, 0.75 mm i.d., 3 µm; Thermo Scientific, San Jose, CA, USA) as the analytical column whereas Easy column C18 (2 cm, 0.1 mm i.d., 5 µm; Thermo Scientific, San Jose, CA, USA) was employed as the pre-column (Kwan et al., 2015).

For the equilibration, the analytical column was run at a flow rate of 3 µL/min for 15 µL, while the pre-column was run at a flow rate of 0.3 µL/min for 4 µL. Meanwhile, the analysis flow rate was set at 0.3 µL/min. Eluent from the sample was sprayed into the mass spectrometer at 220°C capillary temperature and 2.1 kV source voltage. When looking for proteins and peptides, we used a full-scan mass analysis from m/z 300-2,000 with a resolving power of 60,000 (at m/z 400, FWHM; 1-s acquisition) and a data-dependent MS/MS analysis (ITMS) triggered by the eight most abundant ions from a parent mass list of predicted peptides, with rejection or unassigned charge states Collision-induced dissociation with a collision energy of 35 was used for the fragmentation. Each sample was analysed three times for technical replication.

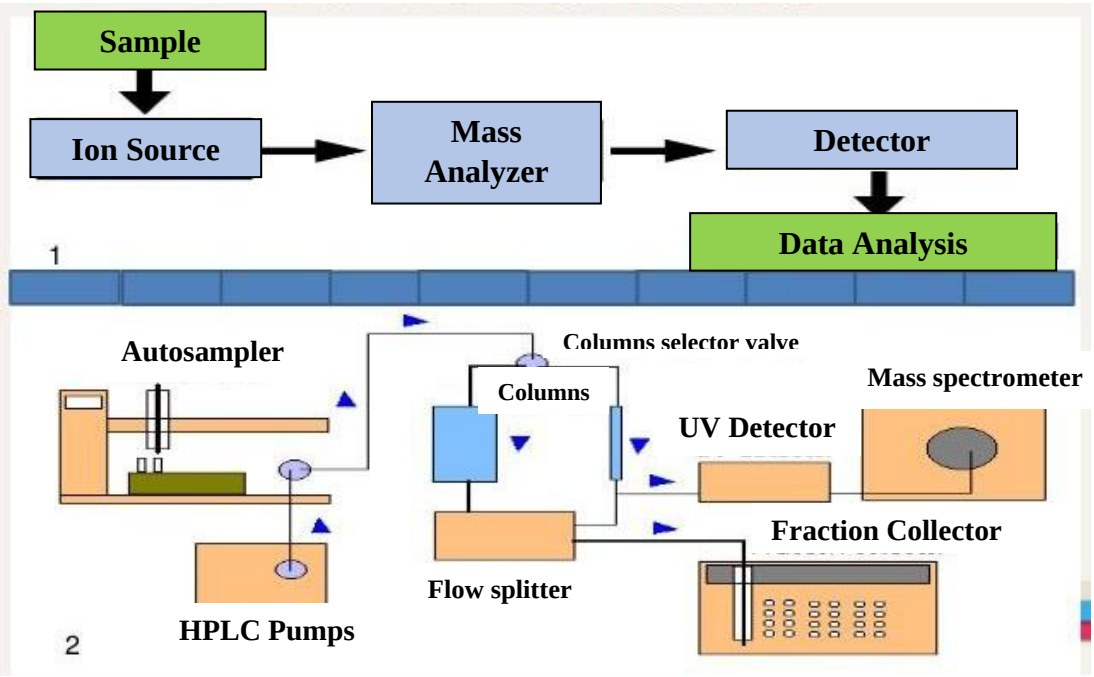


Figure 3.4.6: LC-MS schematic.

Chapter-4: Results

4.1 Calculation of the yield of extracted protein:

The total yield of seaweed protein extract powder obtained from seaweed powder was 33.494±0.709066 %. Percentage of yield of protein extract from *Pyropia umbilicalis* is shown in table 4.1.

Table 4.1: Calculation of the yield of extracted protein.

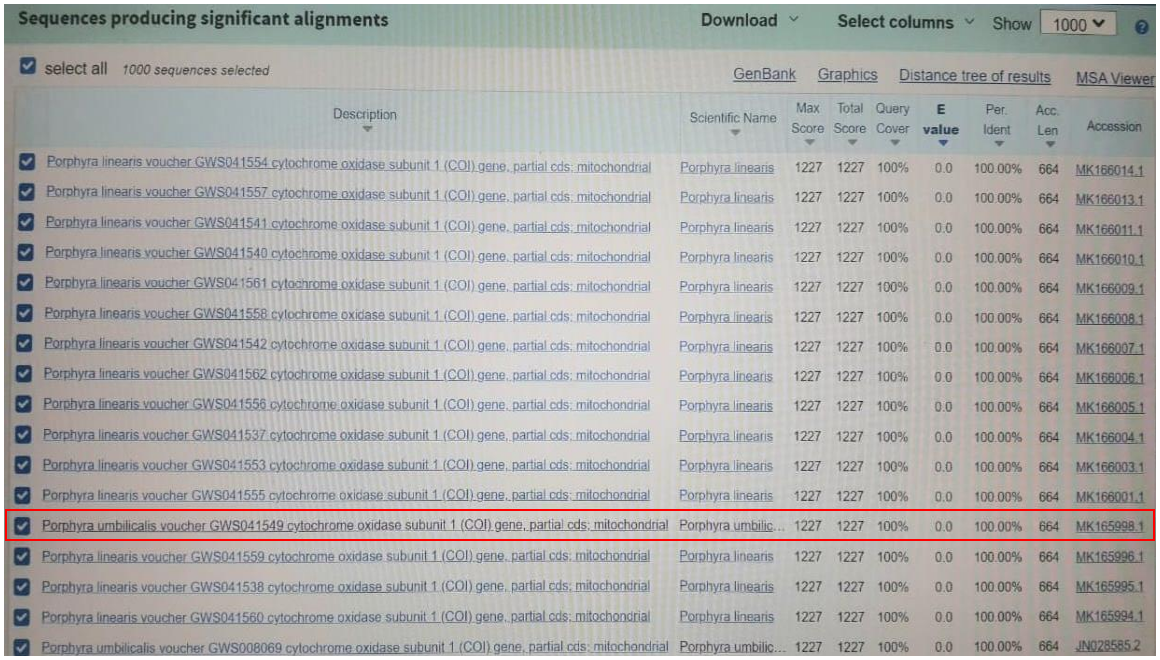
Weight of freeze dried seaweed powder (gm)	Weight of extracted protein (gm)	Percentage of Seaweed protein extract powder (%)	Mean	Standard deviation
30	9.812	32.706	33.49433	0.709066
30	10.224	34.08		
30	10.109	33.697		

4.2 Sample Identification:

The sequence we got provided by the partial DNA test was-

ACTAGCTCAACCAGGCAACCAATTGCTTTTAGGAAATCATCAAATTTATAATG
TATTAGTCACAGAGCATGCTTTTTTAATGATTTTCTTTATGGTAATGCCTGTAT
TAATAGGCGGGTTTGGTAATTGATTTGTACCTATTATGATAGGTGCACCAGAC
ATGGCATTTCCTCGATTAAATAATATTAGTTTTTGATTATTACCCCCATCACTA
TGTCTTCTTTTAGGGTCAGCTATGGTTGAAGTAGGAGTAGGAACAGGTTGAA
CATTATATCCCCCACTAAGTTCTATTCAAAGTCACTCTGGAGGAGCCGTTGAT
CTAGCTATTTTTAGTTTGCATTTATCGGGAGCTTCTTCTATACTTGGTGCTATT
AATTTTATTACTACCATATTCAATATGCGCAATCCAGGGCAAAGTATGTACCG
TATTCCATTATTTGTCTGGTCTATCTTAATTACAGCTTTTCTGTTGTTATTAGC
CGTTCCTGTATTAGCGGGTGCTATTACAATGTTACTCACGGATAGAAATTTTA

ATACCACATTTTTTGATCCTTCAGGTGGAGGTGACCCCGTTTTATATCAACAT TTATTC. Then according to NCBI BLAST, the sequence matched with the sequence of *Pyropia umbilicalis*. NCBI BLAST result is shown in figure 4.2.



Sequences producing significant alignments Download Select columns Show 1000

select all *1000 sequences selected* GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Porphyra linearis voucher GWS041554 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166014.1
Porphyra linearis voucher GWS041557 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166013.1
Porphyra linearis voucher GWS041541 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166011.1
Porphyra linearis voucher GWS041540 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166010.1
Porphyra linearis voucher GWS041561 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166009.1
Porphyra linearis voucher GWS041558 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166008.1
Porphyra linearis voucher GWS041542 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166007.1
Porphyra linearis voucher GWS041562 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166006.1
Porphyra linearis voucher GWS041556 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166005.1
Porphyra linearis voucher GWS041537 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166004.1
Porphyra linearis voucher GWS041553 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166003.1
Porphyra linearis voucher GWS041555 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK166001.1
Porphyra umbilicalis voucher GWS041549 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra umbilic...	1227	1227	100%	0.0	100.00%	664	MK165998.1
Porphyra linearis voucher GWS041559 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK165996.1
Porphyra linearis voucher GWS041538 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK165995.1
Porphyra linearis voucher GWS041560 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra linearis	1227	1227	100%	0.0	100.00%	664	MK165994.1
Porphyra umbilicalis voucher GWS008069 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Porphyra umbilic...	1227	1227	100%	0.0	100.00%	664	JN028585.2

Figure 4.2: NCBI BLAST result.

4.2 Proteins identified by LC-MS mass spectrometry:

The strong cell walls of the red seaweeds are made up of cellulose fibrils that are encased in an enormous gelatinous matrix that is made up of a variety of sulfated galactose polymers. Protein extraction is much more difficult when these fibrillar polymers are present because they bind to the protein. Following the Bradford assay, proteins were isolated from the red algae *Pyropia umbilicalis*, and the total amount of soluble protein was determined to be 0.75±0.002%. Then for mass spectrometric analysis, proteolytic digestion of protein was carried out with the protease trypsin as described in "Materials and Methods." Following that, the material was analyzed using LC-MS/MS technology. The masses of the peptides were queried against the several public protein databases that were readily available. Here I reported 8 peptides (Table 4.3).

Table 4.2: *Pyropia umbilicalis* proteins identified by LC-MS mass spectrometry.

Protein name	Organism Species (OS)	Accession No.	No of peptides matched	Molecular mass (Da)	Coverage (%)
R-phycoerythrin beta chain	*Pyropia tenera*	P84862	12	18,198	53
	Pyropia haitanensis	Q0ZHI8	12	18,423	53
	Agarophyton chilensis	Q7SIF9	4	18,604	19
R-phycoerythrin alpha chain	*Pyropia tenera*	O49843	5	17,666	26
	Pyropia haitanensis	Q0ZHI7	5	17,682	26
	Pyropia yezoensis	O20206	5	17,698	26
	Porphyra purpurea	P51368	5	17,698	26
	Agarophyton chilensis	Q7SIG0	4	17,752	22
B-phycoerythrin beta chain	*Porphyridium sordidum*	P29948	6	18,554	32
	Porphyridium purpureum	P11393	6	18,554	32
C-phycocyanin alpha chain	*Pyropia haitanensis*	Q0ZHI5	5	17,464	36
	Pyropia yezoensis	Q1XDA9	5	17,464	36
C-phycocyanin beta chain	*Pyropia yezoensis*	Q1XDB0	5	18,201	41
	Pyropia haitanensis	Q0ZHI6	5	18,185	41
	Porphyra purpurea	P51377	5	18,198	41
Ribulose bisphosphate carboxylase large chain	*Pyropia haitanensis*	Q760R5	3	54,030	7
	Pyropia dentata	Q760S7	2	54,074	5
	Pyropia yezoensis	Q760T5	2	54,060	5
	Pyropia katadae	Q760R9	2	54,060	5
	Ectocarpus siliculosus	P24313	1	53,905	2
	Pylaiella littoralis	P23651	1	54,124	2
Allophycocyanin beta chain	*Pyropia yezoensis*	P59857	1	17,484	7
	Pyropia haitanensis	Q6UDP8	1	17,454	7
	Porphyra purpurea	P51261	1	17,484	7
Allophycocyanin alpha chain	*Mastigocladus laminosus*	P00315	1	17,121	6
	Cyanidium caldarium	Q9TLS7	1	17,603	6
	Pyropia yezoensis	P59856	1	17,509	6
	Galdieria sulphuraria	P00314	1	17,532	6

Protein name	Organism Species (OS)	Accession No.	No of peptides matched	Molecular mass (Da)	Coverage (%)
Allophycocyanin alpha chain	*Aglaothamnion neglectum*	P28555	12	17,485	6
	Porphyra purpurea	P51262	1	17,509	6
	Cyanophora paradoxa	P00316	1	17,279	6
	Pyropia haitanensis	Q6UDP9	1	17,479	6

4.3 Identification of functional protein:

Eight pure protein found from protein extract of *Pyropia umbilicalis* belong to photsynthetic pigment protein, photorespiration protein and heat shock protein.

Table 4.3: Identification of functional protein.

Protein name	Organism Species (OS)	Functional Group
R-phycoerythrin beta chain	*Pyropia tenera*	Photosynthetic Pigment and Heat Shock Protein
	Pyropia haitanensis	
	Agarophyton chilensis	
R-phycoerythrin alpha chain	*Pyropia tenera*	
	Pyropia haitanensis	
	Pyropia yezoensis	
	Porphyra purpurea	
	Agarophyton chilensis	
B-phycoerythrin beta chain	*Porphyridium sordidum*	Photosynthetic Pigment Protein
	Porphyridium purpureum	
C-phycocyanin alpha chain	*Pyropia haitanensis*	
	Pyropia yezoensis	
C-phycocyanin beta chain	*Pyropia yezoensis*	
	Pyropia haitanensis	
	Porphyra purpurea	

Protein name	Organism Species (OS)	Functional Group
Allophycocyanin beta chain	*Pyropia yezoensis*	Photosynthetic Pigment Protein
	Pyropia haitanensis	
	Porphyra purpurea	
Allophycocyanin alpha chain	*Mastigocladus laminosus*	
	Cyanidium caldarium	
	Pyropia yezoensis	
	Galdieria sulphuraria	
	Aglaothamnion neglectum	
	Porphyra purpurea	
	Cyanophora paradoxa	
	Pyropia haitanensis	
Ribulose bisphosphate carboxylase large chain	*Pyropia haitanensis*	Photorespiration Related Protein
	Pyropia dentata	
	Pyropia yezoensis	
	Pyropia katadae	
	Ectocarpus siliculosus	
	Pylaiella littoralis	

Chapter-5: Discussion

5.1 Protein Identification:

A partial DNA test was performed after collecting the red seaweed sample in order to determine the sample's molecular features. NCBI BLAST analyses revealed that the DNA sequence is similar with *Pyropia umbilicalis*. Recently, Kim et al. (2012) used a partial DNA test to determine the molecular structure of the Korean species *Gelidium elegant*. Recent molecular investigations of Gracilariaceae (Rhodophyta) via DNA tests for identification from the Asia-Pacific region were conducted by Yang and Kim (2015). DNA test it is a reliable tool when species identification is required in a quick manner.

5.2 Total yield of extracted protein:

Protein extraction was done by following Galland-Irmoulin et al. (1999) with slight modification. In this method we use alkaline solutions to extract protein from *Pyropia umbilicalis.* Numerous studies have demonstrated the efficiency of alkaline solution (NaOH) in assisting in the extraction of extremely water-insoluble proteins from seaweed (Amano et al., 1990; Lourenco et al., 2002). The total yield of extracted protein of *Pyropia umbilically* is 33.494±0.709066 %. Using same extraction method recorded values for other seaweed species including *Caulerpa veravelensis* (7.77±0.59%), *Caulerpa scalpelliformis* (10.50±0.91%), *Palmaria palmata* (13.5%) (Kumar et al., 2014). In comparison with data of previous study, the total yield of extracted protein from *Pyropia umbilically* is higher than many of other seaweeds. So, It could be a good protein source.

5.3 Identification of functional protein:

LC-MS/MS analysis and bioinformatics method were used to identify 8 distinct proteins, which are listed in Table 4.4. The light-harvest protein-pigment complex, photosynthetic protein, and heat shock/response protein were the most often discovered proteins. The highest component of all the functions suggested by the discovered proteins is found in the proteins that interact with light harvest pigment. Following this are photorespiration, which includes carbon dioxide fixation, the Calvin cycle, oxidative fragmentation of the

pentose substrate, oxidation-reduction reaction, protein chromosomal linkage, and heat shock/response protein.

5.3.1 Photosynthetic pigment-protein:

Rhodophyta can be identified by the distinctive photosynthetic pigments that make up the phycobiliprotein family, including phycocyanin, phycoerythrin, and allophycocyanins (Campbell and Woelkerling, 1990; Stadnichuk et al., 2015). Phycoerythrin is red (max = 565 nm), allophycocyanin is bluish-green (max = 650 nm), and phycocyanin refers to the blue (max = 620 nm) photosynthetic pigments (Grossman et al., 1993; Rudiger, 2002; MacColl, 1998; Stadnichuk et al., 2015).

Phycobiliproteins, a type of fluorescent proteins covalently linked to tetrapyrrole groups (bilins) as a prosthetic group, account for up to 50% w/w of the total protein composition of red seaweeds (Cian et al., 2015; Niu et al., 2007). They are involved in the absorption of light (in the visible region from 450 nm to 650 nm). They are somewhat close to photosystem II, which is one of the two pigment complexes that are involved in the mechanism of photosynthesis. With the help of this special configuration, chlorophyll can transport light energy with an efficiency of more than 90% (Fleurence, 2003; Talarico, 1996). Therefore, these phycobiliproteins serve as antennas that capture different wavelengths of light . And they're the most common type of functional protein found in *Pyropia umbilicalis*. As sunlight is scarce underwater, these photosynthetic pigments are necessary to maximize the capture of sunlight for photosynthesis.

Additionally, these phycobiliproteins are commonly used as natural colorants in a wide variety of products, including food, cosmetics, printing inks, textiles, and even medicines (Sekar and Chandramohan, 2008). The luminous properties of phycobiliproteins have also made them popular for use in clinical and immunological research (Sekar and Chandramohan, 2008; Spolaore et al., 2006). The biomedical field also depends on these phycobiliproteins (Sekar and Chandramohan, 2008). The antioxidant, anti-cancer, anti-inflammatory, neuroprotective, and hepatoprotective effects of phycocyanin, for instance, have been demonstrated (de Morais et al., 2018; Hussein et al., 2015; Jiang et al., 2017; Kim et al., 2018). Phycoerythrin has anti-aging and anti-Alzheimeric properties (Sonani et al., 2014; Sonani et al., 2017).

Therefore, both proteins are marketed as highly fluorescent dyes in biological and biomedical research, as well as natural functional food supplements and natural dyes in the food products and cosmetics sectors (Ariede et al., 2017; Matos, 2017), and are readily available via a number of well-established and acknowledged extraction methods (Galanakis, 2019). A recent study by Pereira et al. (2018) also suggested that athletes supplement their diets with algal protein. This has led to these proteins being widely distributed and accepted as a type of functional food or nutraceutical.

5.3.1.1 Phycocyanin:

Previous research has shown that phycocyanin has many beneficial effects, including those that are antioxidant, anti-inflammatory, antiviral, anticancer, anti-allergic, anti-mutagenic, immune-enhancing, hepatoprotective, blood vessel-relaxing, and blood-lipid-lowering (Cian et al., 2015; Eriksen, 2008; Madhyastha et al., 2009; Richa et al., 2011; Thangam et al., 2013; Zheng et al., 2013).

Phycocyanin is an antioxidant that also inhibits the production of inflammation-causing compounds (Romay et al., January 1998; Romay et al., 2003). C-PC can remove oxidants such as peroxyl, hydroxyl, and alkoxyl radicals. Peroxyl radicals are also neutralized by C-PC, but to a lesser extent. C-PC inhibits lipid peroxidation and so acts as a metal-binding antioxidant (Romay et al., August 1998). It is the chromophore (a component of C-PC) that stabilizes the peroxyl radicals (Patel et al., 2006). Low light and high concentrations of C-PC are required for hydroxyl radical scavenging (Zhou et al., 2005). Inflamed parts of the body have a higher concentration of hydroxyl radicals (Romay et al., August 1998). C-PC, which is an anti-oxidant, is able to scavenge these radicals that cause damage, and as a result, it acts as an anti-inflammatory agent.

When there is an abundance of oxygen in the brain, reactive oxygen species are produced (ROS). Free radicals (ROS) in the brain cause damage to neurons, which in turn can lead to strokes. In order to reduce oxidative stress, C-phycocyanin is responsible for the removal of the reactive oxygen species known as hydrogen peroxide from the inside of the astrocyte (Min et al., 2015). Astrocytes, due to their ability to boost synthesis of growth factors such as BDNF and NDF, contribute significantly to improve nerve

regeneration. Additionally, astrogliosis and glial inflammation can be prevented by C-PC (Liu et al., 2016; Min et al., 2015).

C-phycocyanin has been shown to protect against hepatotoxicity (Romay et al., January 1998; Vadiraja et al., 1998). By elevating serum levels of glutamic pyruvic transaminase, treatment with C-PC offers protection against heptatoxins like carbon tetrachloride (CCl4). It is through the Cytochrome-P450 system that C-PC provides liver protection (Vadiraja et al., 1998). Scavenging reactive metabolites is a second potential defense mechanism by C-PC (or free radicals if the cause is CCl4).

C-phycocyanin has been shown to possess anti-cancer effects (C-PC). Cancer develops when cells continue to divide and multiply out of control. Researchers have discovered that C-PC can inhibit cell development (Basha et al., 2008). Before the S phase, C-PC inhibits tumor development. Because the tumor cell has entered G0, it will not divide or produce any new tumor cells (Liu et al., 2016). Additionally, C-PC induces apoptosis. ROS, also known as radical oxygen species, are produced after cells have been treated with C-PC. These compounds inhibit the development of BCl-2, a protein that regulates apoptosis. Here, BCl-2 acts to inhibit caspases, which are proteins. Caspases are an integral part of the biological process known as apoptosis. Caspase expression heightens when BCl-2 levels drop.As a result, apoptosis occurs (Liu et al., 2016; Pardhasaradhi et al., 2003). In order to effectively treat cancer, C-PC must operate in conjunction with other medications to overcome the persistant nature of tumor cells.

5.3.1.2 Phycoerythrin:

Red algal phycoerythrin subunits were found to be useful as a photosensitizer in cancer cell photodynamic therapy (Cian et al., 2012). Stress tolerance in 5-day-old individuals was similarly improved by PE therapy, with a rise in the mean survival rate from 22.2±2.5 to 41.6±2.5% under thermal stress and from 30.1±3.2 to 63.1±6.4% under oxidative (hydrogen peroxide)-stress (Sonani et al., 2014). Furthermore, heat-induced production of human amyloid-beta(A1-42) peptide and associated paralysis in the muscular tissues of transgenic C. elegans CL4176 (Alzheimer's disease model) was found to be attenuated by PE therapy (Sonani et al., 2014). Because of its anti-oxidative effect,

phycoerythrin from Porphyra haitanensis has been recently described as a possible medication for cancer prevention (Pan et al., 2013).

5.3.2 Photorespiration related protein:

The second highest function is photorespiration, which is exemplified by the enzyme ribulose bisphosphate carboxylase (RuBisCO). Photorespiration (the oxidative portion of photosynthesis) is an essential part of plant metabolism. This mechanism prevents water loss by closing stomata when a plant is under stress, such as from exposure to high levels of light or heat (Wingler et al., 1999). Since Pyropia umbilicalis lives in the intertidal zone, it may be subjected to dangerously high levels of sunlight during low tides. The photosynthetic system of red algae, a kind of C3 plants, is known to be safeguarded by photorespiration (Heber et al., 1996).

5.3.2.1 Ribulose bisphosphate carboxylase:

RuBisCO is the most common protein on Earth and is recognized as the principal enzyme in both the process of photosynthesis as well as the process of photorespiration in plants and other creatures. Depending on whether it has an affinity for carbon dioxide or molecular oxygen, the multimeric plant metabolic enzyme known as RuBisCO takes part in either photorespiration or carbon fixation in the Calvin cycle (Barbeau and Kinsella, 1988). These two processes are dependent on one another. In order to create organic molecules from carbon dioxide, this enzyme is essential. These organic compounds will be metabolized by the plant to produce sugar.

In addition to being essential for plant physiology, RuBisCo has gained recognition as a functional food due to its many health benefits (Udenigwe et al., 2017). Antibacterial, antihypertensive, and opioid-like effects were found in RuBisCo-derived peptides. RuBisCo's peptides are satiating and prevent diabetes and oxidative stress (Udenigwe et al., 2017). RuBisCO will not be able to be taken seriously as a sustainable source of bioactive peptides in industrial food formulations until additional study has been conducted (Kobbi et al., 2017). Therefore, bioactive peptides derived from RuBisCO are both desirable and sustainable. It is currently possible to extract the RuBisCO subunits using a variety of procedures, some of which are easy, quick, and adaptable to large scale production (Udenigwe et al., 2017).

The blood pressure of hypertensive rats was shown to be reduced by RuBisCO peptides, as reported by Kapel et al. (2006). In the meantime, RuBisCO enzymatic hydrolysates of Significant in vitro antioxidative capacity has been demonstrated by Kobbi et al. (2017), by inhibition of linoleic acid oxidation, reduction of ferric ion, and scavenging of a radical cation (90 per cent purity). The protein has the potential to be used as a functional ingredient in a wide range of other food applications due to the fact that. Increasing food product stability and shelf life can be accomplished by using peptides that are produced from RuBisCO. These peptides can play an important role in mitigating the oxidative damage that can be caused during the manufacturing and storage of food (Aluko, 2018; Udenigwe et al., 2017).

5.3.3 Heat shock protein:

Since pyropia-related rhodophyta normally develop at the intertidal zone, they are frequently exposed to a wide range of abiotic stress conditions (Sahoo et al., 2002). For instance, variations in tide level will lead to temperature fluctuations, heat exhaustion, humidity, and varying light intensities (Hwang et al., 1997; Uji et al., 2019). To survive the ongoing temperature and heat variations, these plants modify the expression of their genes. Proteins like heat shock protein were therefore developed (HSP). Basha et al. (2012) found that these HSP genes were turned on whenever the plant was under stress. HSPs also serve an important function in stabilizing proteins and membranes under stress by binding to proteins and encouraging protein refolding (Vabulas et al., 2010). Phycoerythrin extracted from *Pyropia umbilicalis* is a heat shock proein. Up to 40°C, phycoerythrins shown impressive thermal stability (Munier et al., 2014). Galland-Irmouli et al. (2000) also found that *Palmaria palmate*'s R-PE was stable up to 60°C. The 495 nm peak also showed a great stability toward high temperature.

According to Uji et al. (2019), the HSPs found in plants and other species can be broken down into five groups depending on their molecular sizes: Hsp100, Hsp90, Hsp70, Hsp60, and tiny HSPs (sHSPs/HSP20). Several studies have been conducted to isolate and characterize the HSPs from red seaweeds of the genus Pyropia (Sun et al., 2016; Uji et al., 2019). Additional research investigated how HSPs function as molecular

chaperones, folding, assembling, localizing, secreting, and translocating cellular proteins while normally being expressed at low levels (SINGH et al., 2016).

Colaco et al. (2013) conclude in their review that HSPs play a crucial role in immunology. Such HSPs have a history of serving as vital immunogens in the body's fight against infections (Osterloh and Breloer, 2008; Suzue et al., 1997). HSPs have critical roles in a variety of diseases and ailments, including apoptosis, neurological disorders, and gastrointestinal issues. HSPs can be used as novel molecular targets in pharmacological and therapeutic methods for the treatment and prevention of a wide variety of diseases (SINGH et al., 2016). Moreover, they provided evidence that HSPs served diagnostic, anti-inflammatory, and cancer-fighting roles (Kundapur and Kumar, 2015).

Chapter-6: Conclusion

Maintaining food security in the face of rising global population, decreasing farmland, and unpredictable weather patterns requires urgent action. Protein is an essential macronutrient for human health, therefore making sure we get enough of it in our diets is a top priority. Animal proteins are good sources of dietary protein, however the manufacturing of animal proteins results in a large carbon footprint. Because of this, there has been an attempt to find alternative protein sources. The purpose of this research was to provide a more in-depth look at the methods used to extract the proteins from the red seaweed *Pyropia umbilicalis* and to investigate the potential of this algae as a protein substitute. It was found eight distinct protein with three main functional activities which are are as follows R-phycoerythrin beta chain, R-phycoerythrin alpha chain, B-phycoerythrin beta chain, C-phycocyanin alpha chain, C-phycocyanin beta chain, Ribulose bisphosphate carboxylase large chain, Allophycocyanin beta chain, and Allophycocyanin alpha chain. The functional properties of these proteins could make them useful in numerous dietary applications, and their bioactivities could be modified to serve a variety of nutraceutical functions. In recent years, the utilization of proteins extracted from red seaweed as functional food additives is emerging with promising applications in bioactive microencapsulation. Bringing seaweed farming to a commercial scale and winning over western consumers will require significant improvements to the current cultivation technique. Extending the efficiency of seaweed protein extraction and expanding its uses in the food and nutraceutical industries both require additional study.

Chapter 7: Recommendations and Future Perspective

The model red seaweed proteome developed in the present study could be used to compare and distinguish between closely related species. Such information is a prerequisite for future research on seaweed marker proteins for species identification to aid in taxonomic examination, identification of halotolerant proteins, indicators of heavy metal contamination, and proteins essential to the survival of the species. The seaweed, *Pyropia umbilicalis* protein extract needs to be the subject of more research in order to produce pharmaceuticals, functional products, or value-added products from this seaweed protein. Study on developing pharmaceutical products incorporating sea-weed protein extract to increase their market value and to provide enhancement to human health. Study on fractionation and purification of digestive enzymes from seaweed protein extract can be implemented. Research should be done to identify the active components particularly DPP-IV and ACE inhibitory peptides of the seaweed protein extract. Seaweed protein extract should be studied for its potential to generate bioactive peptides with antioxidant, anti-diabetic, and antibacterial activities. The outcomes of this study will be beneficial for more in-vitro and in-vivo research.

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Appendix A: Photo Gallery

Raw sample

Freeze dried sample

Weighing of dried sample

Preparation of acetate buffer

Seaweed powder added into buffer

Weighing of cellulase enzyme

Enzyme added into buffer solution

Mixture of buffer, seaweed powder, and enzyme

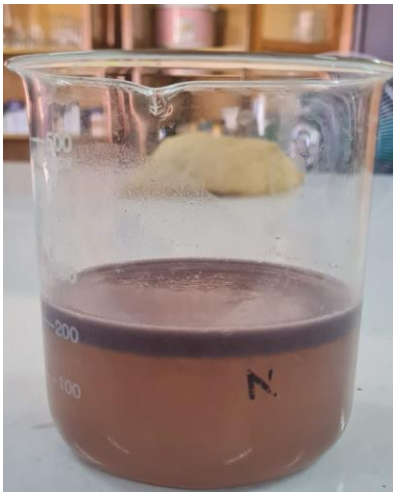
Magnetic stirring

Water bath

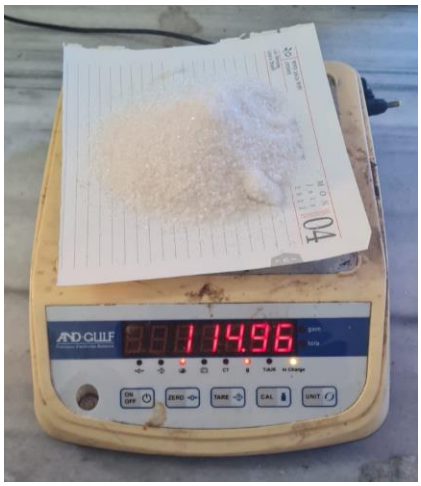
Sonication

Sample after sonication

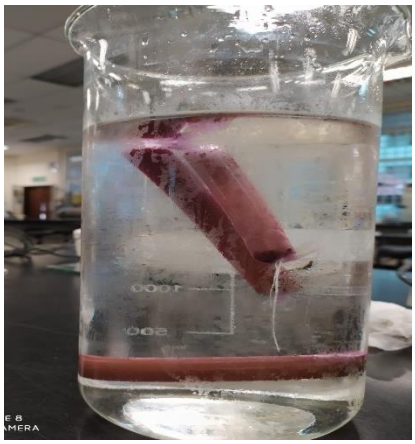
Centrifugation



Supernatant after Centrifugation



Weighing of ammonium sulphate



Dialysis

Brief Biography

Jannatul Farhana passed the Secondary School Certificate Examination in 2011 from Narayanhat Collegiate High School, Chattogram, and then Higher Secondary Certificate Examination in 2013 from Chattogram Govt. Model College, Chattogram. She completed her B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving health status of people through proper guidance and suggestions and to create awareness among people about food safety and nutrition.