Determination of Nutritional Composition of Potential Red Seaweed (*Porphyra umbilicalis*)

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Roll No: 01-21/09

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Department of Applied Food Science and Nutrition

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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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List of Abbreviations

AA	Amino Acid
AOAC	Association of Official Analytical Chemists
BC	Before Christ
CHO	Carbohydrate
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
EAA	Essential Amino Acid
FAO	Food and Agricultural Organization
GAE	Gallic acid equivalent
GHG	Green House Gas
HPLC	High Performance Liquid Chromatography
IPCC	Intergovernmental Panel for Climate Change
LDL	Low Density Lipoprotein
NEAA	Non-Essential Amino Acid
Nitro-PAPS	2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamine) phenol
PUFA	Poly Unsaturated Fatty Acid
RDI	Recommended Daily Intake
spp.	Species
sPS	sulfated Polysaccharides
SPSS	Statistical Package for Social Science
TCC	Total Carotinoids
QE	Quercetin equivalent

Abstract

Since ancient times, seaweeds are considered as a natural source nutrients and bioactive compounds. Scientist's believe that, they can be used as a functional ingredient in many technological applications to obtain functional foods. In order to obtain more nutritional information of the locally available seaweed species- *Porphyra umbilicalis*, chemical composition, amino acid contents, as well as some physicochemical properties were measured in this investigation. It was discovered that the percentage of carbohydrate content was almost half of the total nutritional composition (49.32± 0.67%). 21.60±0.44% protein, 16.42±0.47% ash, 6.06±0.50% moisture and 5.44±0.01% crude fiber was found in the collected sample. Very low percentage of lipid content (0.50±0.25%) was found in the sample. Among the twelve amino acids, the highest values for amino acids were found for valine, threonine, isoleucine, and phenylalanine. The fatty acid profiles were dominated by the polyunsaturated fatty acids (55.96%) particularly following saturated fatty acids (34.75 %) and monounsaturated fatty acids (9.29%). A very low concentration of vitamin A and vitamin E was found from the collected samples. Higher amount of Na, K, Mg, Ca, content were found from 100g of seaweed sample compared to the concentration of Zn and Fe. The physicochemical properties of this seaweed namely the water holding and the swelling capacity were comparable to some commercial fibre rich products. This study suggested that the selected red seaweed could be potentially used as raw materials or ingredients to improve the nutritive value and texture of functional food and healthy products for human beings.

Keywords: Red seaweed, macro algae, nutritional composition, physicochemical properties, significant value

Chapter-1: Introduction

Humans generally rely on livestock and seeds of terrestrial plants for sources of high-quality protein. The increased use of farmland and freshwater, however, has negative effects on the climate and jeopardizes the viability of the world's food systems (Springmann et al., 2018). Food systems were responsible for 35% of all anthropogenic greenhouse gas (GHG) emissions, with animal-based diets accounting for more than half of this footprint (Xu et al., 2021). Therefore, one of the most viable strategies for achieving global sustainability is to look for sustainable food supplies, particularly alternative protein sources, in response to the anticipated global food crisis and other environmental and economic issues.

Seaweeds have been studied more and more in a variety of contexts (FAO, 2016), as they are linked to advantageous biological activities (either as a whole or as individual components or extracts) that have supported their use in a number of industries, such as agriculture (Baweja et al., 2016), biofiltering, biofuel (Kraan, 2016), the textile industry (Dawes, 2016), as cosmetics and hygiene products (Rupérez et al., 2002; Couteau & Coiffard, 2016), in thalassotherapy (Baweja et al., 2016), medicine and the pharmaceutical industry (Holdt &Kraan, 2011; Levine, 2016), biotechnology and microbiology (Holdt and Kraan, 2011), as well as in the animal (Pereira, 2015) and human food industries (Holdt and Kraan, 2011)). A significant application is food production, which can be broken down into three categories: dietary supplements, food additives, and edible seaweeds (full intake) (Bixler and Porse, 2011). Seaweeds also have the potential to be functional foods because it has been shown that they provide health advantages that go beyond simple nutrition (Holdt and Kraan, 2011; Mendis and Kim, 2011). Due to their distinctive makeup of bioactive compounds (such as R-phycoerythrin, agar, and carrageenan) and beneficial effects (Mohamed et al., 2012), as well as their increased uses in the food industry (Bixler and Porse, 2011), red seaweeds (phylum Rhodophyta) are of particular interest. Asians are the biggest consumers of seaweed. The production of seaweed grown worldwide in 2019 was 34.7 million tons, or almost $14.7 billion. From this total, red seaweed (Rhodophyta) accounted for 52.6% of the value, or more than USD 7.1 billion, with

Kappaphycus/Eucheuma, Gracilaria, and Porphyra being the three most prevalent cultivated species.

The oldest eukaryotic algae (macroalgae) known to exist in freshwater and marine habitats are thought to be red seaweeds. With over 6500 species found so far, they are the taxonomically most diverse group (Nan et al., 2017). Their unusual coloring, caused by the phycobiliproteins utilized as natural colors in the food industry, is one distinguishing characteristic (Dumay et al., 2013). In Asia, where seaweeds are prized as a delicacy food and used in a variety of recipes, farming for red seaweed is done offshore, primarily along coastlines (Titlyanov & Titlyanova, 2010). Of these, Porphyra, often called Nori, is most frequently used as sushi wraps, appetizers, and a soup ingredient. Red seaweed is a desirable source of protein due to its high levels of utilizable protein (up to 47%) and essential amino acids (EAAs). They typically offer low calorie value but high protein and dietary fiber along with necessary vitamins, minerals, and bioactive components (Gamero-Vega et al., 2020), even though the nutrients are changed by species and environment. Red seaweed has also been found to have a wide variety of possible bioactive peptides (Admassu et al., 2018a), indicating their adaptability as a cheap, nourishing, and sustainable marine source.

One of the most popular and consumed seaweed species in Ireland is *Porphyra umbilicalis*, popularly known as purple laver. It is typically eaten dry and uncooked. Nori (*Porphyra umbilicalis*), which resembles lettuce in appearance, is typically a deep red to purple color. The color of red seaweed species is caused by red phycobilin pigments (phycoerythrin and phycocyanin), which conceal the green pigments chlorophyll-a and beta-carotene (Sánchez-Machado et al., 2004). Sulphated galactans (agar and carrageenans), which make up about 70% of the cell wall constituents, are polysaccharides (SDF) present in red seaweeds. In previous studies, specimens of Porphyra spp. collected from the Atlantic European coast were found to be rich in proteins (Marsham et al., 2007), carbohydrates (Morrissey et al., 2001), minerals (Marsham et al., 2007), vitamins (Morrissey et al., 2001), ω-3 polyun- saturated fatty acids (Sánchez-Machado et al., 2004), glycolipids, phospholipids (Fleurence et al., 1994) and phenols (Rupérez and Saura-Calixto, 2001). Studies on *P. umbilicalis*'s advantageous properties have revealed strengthened skin barrier

function, cell membrane structure restoration, increased epidermal hydration, and antifungal (Corato et al., 2017), photoprotective (Guinea et al., 2012), antioxidant (Cofrades et al., 2010) and anti-inflammatory properties. This red seaweed species is available in the coastal region of Bangladesh, Cox's Bazar. However, *Porphyra umbilicalis* is still underutilized in our country and no research has been done on the nutritional profile of this species.

In order to learn more about *Porphyra umbilicalis* nutritional value, this research seeks to present an in-depth analysis of the chemical make-up, amino acid content, and element content of specimens taken from the Cox bazar inani rock beach and patenga sea beach. This research also looked into a few physicochemical characteristics to assess how they might affect the physiological consequences of functional and healthy foods. For the purpose of giving the food business and scientific community the most recent knowledge, the red seaweed protein extraction methods were also thoroughly evaluated and summarized. Applications of red seaweed now and in the future in particular application fields, sustainability considerations, safety issues, consumer acceptance, and prospective changes to protein functionality were also highlighted.

Aims and Objectives

i. To analyze nutritional composition (protein, lipid, carbohydrate) of seaweed samples (*Porphyra umbilicalis*) collected from inani rock beach and and patenga sea beach in Cox bazar coastal region.
ii. To determine the amino acid and fatty acid content of collected samples.
iii. To assess the concentration of vitamin A as carotenoids and vitamin E as α-tocoferol in *Porphyra umbilicalis.*

Chapter-2: Review of literature

2.1 History of Seaweed

Thousands of different types of microscopic, multicellular, marine algae are referred to as seaweed or macroalgae. For both food and non-food purposes, seaweeds have been used all over the world for thousands of years. For more than 2000 years, seaweed has been consumed as food in China, Korea, and Japan (Brijesh and Declan, 2015). In Japan, dried seaweed sheets called "nori" (*Porphyra* species) are made from seaweed and used to make sushi. Seaweeds are consumed fresh as a salad in Malaysia and Indonesia. The use of seaweed in cuisine has a long history in South East Asian countries, although in the west, it has typically been used for non-food purposes. Seaweeds were used as animal food in Greece as early as 100 BC. Red seaweeds have been utilized as medicine in Mediterranean nations. Farmers in Ireland and Scotland employed seaweed for agricultural purposes, such as soil mulch. In Asia, seaweeds are grown for a variety of uses, although harvesting natural stocks is the most popular method for seaweeds in Europe.

Growing seaweed for food, as a source of chemicals (such carrageenan), as animal feed, and as fertilizer has become a widespread agricultural activity in recent years. Recent research has focused on seaweed cultivation as a potential climate change mitigation strategy for biosequestration of carbon dioxide, along with other advantages like nutrient pollution reduction, increased habitat for coastal aquatic species, and reducing local ocean acidification (Duarte et al., 2017). This is because seaweeds are crucial to marine ecologies and for absorbing carbon dioxide. As a mitigation strategy, "further research attention" is advised by the IPCC Special Report on the Ocean and Cryosphere in a Changing Climate (Bindoff et al., 2019).

2.2 Red Seaweed

The multicellular macroalgae Rhodophyta (red), Phaeophyta (brown), and Chlorophyta (green) comprise seaweed. One of the oldest families of eukaryotic algae is the red algae, or Rhodophyta, which is derived from the Ancient Greek words for "rose" and "plant" (Lee, 2008). With about 7,000 currently known species and ongoing taxonomic revisions, the

Rhodophyta is one of the largest phyla of algae (Guiry & Guiry, 2016). The Florideophyceae (class) has 6,793 species, the bulk of which are multicellular marine algae, including many well-known seaweeds (Guiry and Guiry, 2016; Thomas, 2002). Red algae are common in coastal areas but are scarce in freshwater environments (Dodds, 2019).

Figure 2.2: *Porphyra* sp.

The red algae make up a distinct group that is distinguished by having eukaryotic cells without centrioles and flagella, chloroplasts without external endoplasmic reticulum and stroma-containing thylakoids, and using phycobiliproteins as accessory pigments to give them their red color (Woelkerling, 1990). However, despite their name, they can have a wide range of colors, including bright green, soft pink, hues of red and purple, resembling brown algae, and, at deeper depths, virtually black. As food reserves outside of their plastids, red algae store sugars as floridean starch, a kind of starch composed of highly branched amylopectin lacking amylose. Additionally, most red algae are multicellular, microscopic, marine, and sexually reproducing (Viola et al., 2001). Rather than having two generations, the red algal life history typically alternates three generations. This place is for the coralline algae, which secrete calcium carbonate and are crucial to the development of coral reefs. Red algae are used to manufacture agar, carrageenans, and other food additives. Red algae include dulse (*Palmaria palmata*) and laver (nori/gim), which are classic ingredients in European and Asian cuisines (Guiry, 2007).

2.3 Chemical Composition of Seaweeds

Table 1 lists the proximate composition of various algal species. Seaweeds that have been air dried typically include 3 to 47% of carbohydrates, 33 to 75% of proteins, 1.5 to 4% of lipids, and 10 to 35% of ash, respectively. Fresh seaweeds often contain between 80% and 90% of moisture, compared to between 10% and 20% for air-dried algae. In general, green and red seaweeds have larger protein concentrations (10–47% of dry weight—DW) than brown seaweeds (5-24% DW); -3 and -6 polyunsaturated fatty acids (PUFAs) make up a substantial portion of the lipid profile of seaweeds (from 0.79% to 7.87% dry matter)

(Denis et al., 2010; Peña-Rodrígue et al., 2011; Yaich, et al., 2011; Gómez-Ordóñez et al., 2011). The species, geographical origin or region of culture, seasonal, environmental, and physiological fluctuations, the time of harvest, the water temperature, and the processing techniques all affect the chemical and nutrient composition of marine seaweeds (Alvarez et al., 2006).

Table 2.3 Chemical composition of different algae (g/100 g dry weight)

Chlorophyta				
Caulerpa lentillifera	9.26 ± 0.03	1.57 ± 0.02	22.20 ± 0.27	[23]
Ulva clathrate	27.2 ± 1.1	2.2 ± 0.1	27.5 ± 0.2	[15]
Ulva lactuca	8.46 ± 0.01	7.87 ± 0.10	19.59 ± 0.51	[16]
Rodophyta				
Chondrus crispus	27.2 ± 1.4	2.0 ± 0.1	21.1 ± 0.1	[24,25,26]
Garateloupia turuturu	22.9 ± 2.0	2.6 ± 0.1	18.5 ± 0.6	[14]
Jania rubens	11.28 ± 0.10	2.05 ± 0.09	44.03 ± 0.45	[27]
Porphyra/Pyropia spp.	26.6 ± 6.3	2.1 ± 1.2	20.6 ± 0.2	[19,24]
Phaeophyta				
Fucus vesiculosus	12.99 ± 0.04	3.75 ± 0.20	20.71 ± 0.04	[28]
Laminaria spp.	6.3 ± 3.8	1.0 ± 0.3	37.6 ± 0.4	[19,24]
Sargassum fusiforme	10.9 ± 1.0	1.4 ± 0.1	-	[19]
Undaria pinnatifida	18.9 ± 9.8	4.5 ± 0.7	39.3 ± 0.2	[19,24]

2.4 Nutritional Value of Seaweeds

Minerals and trace elements are abundant in seaweed. In contrast to most other foods, it frequently has higher concentrations of these nutrients. varied forms of seaweed will have varied levels of minerals since the nutritional value of seaweed changes depending on where it grows and what type it is. The majority of seaweeds contain vitamins (A, B1, B2,

C, E, and K), calcium, folate, potassium, iron, manganese, and copper, among other nutrients.

In the form of certain vitamins (A, C, and E) and protective pigments, seaweed contains a large number of antioxidants. It contains a respectable amount of iodine, a trace mineral essential to the thyroid's wellbeing and operation. Additionally, some seaweeds, such purple laver, include a healthy level of B12. The following nutrients are typically present in every 100 grams of seaweed: 10 grams of carbs 2 grams of protein 1 gram of fat Fiber optics: 35% RDI 80% magnesium RDI : 80% vitamin K Manganese RDI: 70% RDI 65% iodine Sodium RDI: 70% Calcium RDI: 60% 50% RDI for folate RDI 40% potassium RDI Iron: 20% RDI Omega-3 and omega-6 fatty acids, vitamins A, C, and E, phosphorus, B vitamins, and choline are all present in trace levels. An abundant source of antioxidants is seaweed. Additionally, it includes a lot of sulfated polysaccharides (sPS), which are advantageous plant chemicals thought to provide health advantages.

2.5 Properties of Seaweeds

2.5.1 Antiviral Activity

Some sulphated polysaccharides from red algae have reportedly been shown to exhibit anti-viral effect against viruses that cause human infection. The majority of seaweeds, in particular *Aghardhiella tenera* and *Nothogenia* fastigiated, meet the condition that an antiviral polysaccharide have a very low cytotoxic effect on mammalian cells (De Clercq, 1996). Carrageenan has the potential to have antiviral effects in vitro. Human cytomegalovirus and HIV are both very resistant to the antiviral effects of fucoidan (Malhotra et al., 2003; Majczak et al., 2003; Ponce et al., 2003). According to Baba et al. (1988), fucoidan exhibits antiviral properties by preventing virus particles from attaching to host cells. Additionally, it has the ability to prevent sperm from adhering to the zona pellucida (Oehninger et al., 1991).

2.5.2 Antibiotic Activity

Numerous chemicals found in macroalgae have antibacterial properties. Halogenated chemicals including halogenated alkanes, haloforms, alkenes, alcohol, aldehyde, hydroquinone, and ketone are among the intriguing list of substances found in macro algae

(Lincoln et al., 1991). Antibiotic properties can be found in substances including sterols, heterocyclic, and phenolic chemicals. While many of these substances have cleaning and antiseptic properties, there in vivo antibacterial action is frequently only reached at hazardous concentrations (Lincoln et al., 1991). Sterols, heterocyclic, and phenolic chemicals, among others, occasionally exhibit antibacterial properties. These characteristics may be used to create antiseptics and cleaning agents, but the ability to act as an antibiotic in living organisms can only be attained at toxic concentrations.

2.5.3 Anti-Inflammatory Activity

Macroalgae, particularly red seaweeds, are abundant in polyunsaturated fatty acids (PUFAs) with 20 carbon atoms, primarily eicosapentaenoic and docosahexanoic (Gerwick and Bernart, 1993). The two primary byproducts of the oxidative metabolization of C20 PUFAs in seaweeds are prostaglandin and gracilariales. The metabolized PUFAS metabolites in many red algae are known as oxylipins, which are similar to eicosanoids in higher plants and animals and perform physiological functions (Gerwick et al., 1993; Imbs et al., 2001). Eicosanoid and its derivatives are receiving much greater interest in research because of its anti-inflammatory medications (Jacobs et al., 1993), and the abnormal production of these molecules causes a range of diseases associated to inflammation (Gerwick and Bernart, 1993). Leukotriens and hydroxyleicotetraenoic acid are eicosanoids that have been linked to a number of mammalian disorders. They also exhibit some physiologically active qualities such the chemoattraction of netrophills or smooth muscle cells. In obstetrics and gynecology, where it is employed as a cervical dilator, the combined effects of prostaglandins and the expansion of Laminaria stipes are well recognized (Blumenthal, 1988; El-Refaey and Templeton, 1995; Lee et al., 1998).

2.5.4 Anti-Thrombic and Anti-Coagulant Activity

Fucoidan exhibit anti-thrombotic and anti-coagulant properties that are heparin-like in both vivo and in vitro and are mediated by blood coagulation inhibitors like heparin cofactor II or anti-thrombin III (Colliec et al., 1991; Matou et al., 2002). Sulphated fucan has a number of advantages over heparin, including concentration-dependent inhibition of thrombin produced from platelets and concentration-dependent inhibition of thrombin-induced

platelet aggregation. It also lacks the hypotensive effect of thrombin and reduces the sticking of polymorph nucleated leucocytes from rabbit aorta (Trento et al., 2001).

2.6 Bioactive Compounds of Seaweed

2.6.1 Fatty Acids

For healthy cell activity, fatty acids having two or more methylene interrupted double bonds are beneficial, and they are now being used in nutraceutical and medicinal fields. Western society uses fatty acids most frequently to combat obesity and cardiovascular issues as a result of a better understanding of their biological uses (Gill and Valivety 1997; Sayanova et al., 2004). Additionally, PUFAs-which stand for polyunsaturated fatty acids-play a crucial part in the metabolism of cells and tissues. They also regulate membrane fluidity, oxygen and electron transport, as well as heat tolerance (Funk, 2001).

2.6.2 Sterols

Sterols, the most significant chemical component of microalgae, are the main nutrient present in seaweed. Sterols predominate in plants, animals, and fungus, with "cholesterol" being the most well-known sterol found in animals. Animal cell membrane fluidity and cellular function are both impacted by cholesterol, which also serves as a secondary messenger in embryonic signaling. Precursors to steroid hormones and fat-soluble vitamins include cholesterol. Different seaweed species contain different sterols. According to Sanchez-Machado et al. (2004) and Whittaker (2000), red seaweeds include sterols like desmosterol, cholesterol, sitosterol, fucosterol, and chalinasterol. Red seaweeds (Palmaria and Porphyra) have desmosterols that range in content from 87 to 337 g/g dry weight, or 87% to 93% of the total sterol content.

2.6.3 Carotenoids

The most prevalent colours in nature, carotenoids are present in all algae, higher plants, and photosynthetic microorganisms. They stand in for red, orange, or yellow wavelength photosynthetic pigment. The carotenoid pigment varies among different types of seaweed. Carotene, luetin, and zeaxanthin are the primary nutrients found in red seaweeds

(Solomons and Bulux, 1994; Polivka and Sundström, 2004 Ultrafast). Carotenoids are crucial components of the photosynthetic machinery and play a significant part in the photosystem's reaction hub. According to their biological and chemical activities, some carotenoids are also categorized as vitamins (Zhang, 1999).

2.6.4 Polysaccharide

Mycopolysaccharides, storage polysaccharides, and cell wall structure make up the majority of the polysaccharides found in marine algae (Kumar et al., 2008; Murata and Nakazoe, 2001). They are used in a wide variety of products, including stabilizers, emulsifiers, thickeners, feed, and food and drinks (McHugh, 1987; Tseng, 2001). Ulva, a kind of green seaweed, has a dry weight content of 65% polysaccharide. Ascophyllum, Porphyra, and Palmaria are the other seaweed species that have significant polysaccharide concentrations (Holdt and Kraan, 2011).

2.6.5 Dietary Fibers

In comparison to higher plants, edible seaweeds have substantially higher levels of total fiber, which range from 33% to 62% of the dry weight (Lahaye, 1991; Dawczynski, et al., 2007). There has been a lot of interest in seaweed meal, functional foods, and nutraceuticals for human consumption because polysaccharide exhibits antitumor, antiherpetitic bioactivity, anticoagulant, decrease LDL that is low density lipid-cholesterol in rats, antiviral activity, and they protect against obesity, large intestine cancer, as well as diabetes (McHugh, 2003). Amano et al. (2005), Jeon et al. (2005), Lee et al. (2004), and Ghosh et al. (2009)). Seaweeds' undigested polysaccharides are a significant source of dietary fiber, even if they alter how easily dietary protein and minerals are digested (Urbano and Goñi, 2002).

2.6.6 Phycocolloids

Seaweeds' cell walls are made up primarily of phycocolloids, which also play a role in the detection of pathogens in seaweeds (Potin, 1999). It is a high molecular weight polysaccharide made up of sugar unit polymers. Alginates, carrageenan, and agar are the

three primary types of phycocolloides, and they are mostly used in the food and cosmetic industries (Mayer and Lehmann, 2001; Mayer and Hamann, 2004; Smit, 2004).

2.7 Antioxidant Properties of Seaweed

Seaweed has developed robust antioxidant defense mechanisms in response to the highly oxidative circumstances in which they thrive. Algae are similarly exposed to light and high oxygen levels to photosynthetic species, allowing the generation of free radicals and other potent oxidizing agents (Rozema et al., 2002). But the fact that its chloroplasts' thylakoid membranes show no signs of oxidative damage shows that their cells have strong defense mechanisms (Matsukawa et al., 1997). As a result, a variety of bioactive substances, including polyphenols, sulfated polysaccharides, unsaturated lipids, peptides, and amino acids, which have several antioxidant capabilities, are present in seaweeds' chemical makeup. The most prominent of them are carotenoids, fucoidans, and phlorotannins (Jiménez-Escrig, et al., 2001; Hermund et al., 2018). Some seaweeds create a lot of secondary polyphenolic metabolites. The food industry may be able to replace synthetic antioxidants with phlorotannins, which have significant antioxidant capability connected to their structure (Hermund et al., 2018). Due to the substantial diversity in branching positions between their phloroglucinol units (PGUs), these bioactive chemicals, which are commonly found in brown algae, have a large number of isomers. Phlorotannins' antioxidant action appears to be caused by the availability of hydroxyl groups in their structure and the oligomerization of PGUs (Hermund et al., 2018). Additionally, seaweeds have polyphenols that have particular biological properties and that can modify gene expression (Wang et al., 2009; Rodrigo et al., 2011). The capabilities of polyphenols associated to the prevention of aging and cardiovascular and cancer disorders are therefore of great scientific interest (Stagos et al., 2012). Seaweeds include carotenoids, which are effective antioxidants (Hermund et al., 2018). Xanthophyll and tocopherols are the two carotenoids that are most prevalent. Tocopherols are frequently employed in the food business due to their effective radical scavenging action, whereas Xanthophylls—of which Fucoxanthin is the most notable—are effective quenchers of singlet oxygen.

2.8 Potential Health Benefits of Seaweed

Over the past ten years there has been an increase in the scientific community's understanding that food may have positive physiological and psychological impacts in addition to basic sustenance. On the basis of this assumption, a coordinated effort known as "Functional Food Science in Europe" (Diplock et al., 1999) was launched with the aim of developing scientific methods to characterize the extranutritional benefits of foods, in order to enhance health and well-being and/or lower the risk of disease. Functional foods represent a new category of remarkably promising products whose properties (including the ability to decrease plasma cholesterol, counteract aging through antioxidant mechanisms, etc.) have already made them very popular (Arvanitoyannis et al., 2005; Ashwell, 2002).

Figure 2.8. Biological properties of seaweed

Because of their heterogeneous composition, algae may improve some body functions and/or reduce the risk of certain diseases. According to the studies reviewed and the effects reported, as well as to the approach adopted by the “Process for the Assessment of Scientific Support for Claims of Foods (Howlett and Short, 2004) the principal benefits of

alga consumption can be associated with cardiovascular and intestinal health, while a possible link to bone health and body weight regulation cannot be discarded.

The general margin of health benefits of seaweed include:

- Improves thyroid function
- Improves cardiovascular and gut health
- Stabilizes blood sugar levels
- Boost immune health and may reduce cancer risk

2.9 Functional Food Application of Seaweed

Traditionally, seaweeds are used as folk medicine for treating diseases like goiter, wounds, burns, rashes, inflammation, diabetes and also gaining attention of pharmaceutical industries due to their anti-cancer, anti-aging, anti-angiogenesis, anti-bacterial, anti-viral and antioxidant properties. Seaweeds polysaccharides have wide applications in foods as well as in pharmaceutical industry due to their biochemical properties such as stabilizer, emulsifier and gelling property.

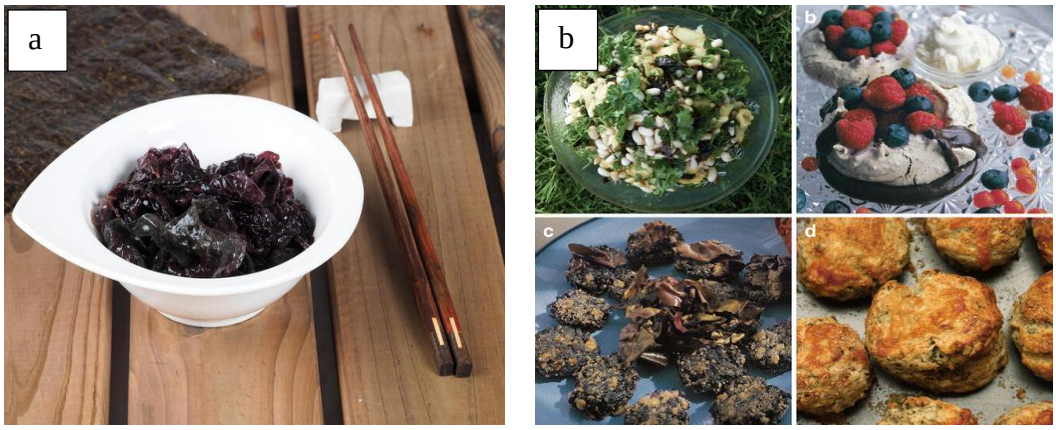

Figure 2.9. Seaweed based dishes; raw (a) and cooked (b)

In food industry, seaweed polysaccharides are used as a functional ingredient in many products such as frozen foods, ice-cream, jam, jelly, beverages etc. Several commercial food preparations from seaweeds are also available in the market such as sea salt, nori snack wasabi, pink rock salt, seaweed thins toasted coconuts, crunchy seaweed chips, and raw unroasted seaweed under different brand names.

2.10 World production of seaweed and its economic significance

Marine plants have been exploited for centuries by coastal communities (Ergueta-Martínez, 2001) In this regard, marine algae are extremely versatile products of the sea that can be consumed directly or as ingredients in many dishes. At present, the seaweed industry offers a wide range of compounds (Buschmann et al., 2001) to meet the increasing demands of the textile, cosmetics, and food sectors. Seaweed products are of particular importance in the food industry (Jensen, 1993) which utilizes them as components of fertilizers, in animal feed supplements (Indergaard M, Minsaas J, 1991) and as additives for human food (Jiménez-Escrig and Goñi, 1999). Industrially cultivated seaweed now supplies more than 90% of the algae marketed internationally (Griffin et al., 1999) Seaweeds are presently cultivated in 35 countries (FAO, 2006) of which the principal producer is the People's Republic of China, followed by Japan and the Republic of Korea. The demand for algae in these countries is the basis of the worldwide industry that produced a harvest worth between $5,500 and $6,000 million U.S. in 2004. Of the total benefits, $5,000 million U.S. were from food products for human consumption, while a large percentage of the remaining profits derived from the industrialization and commercialization of hydrocolloids (FAO, 2006).

2.11 Current Status of Seaweed Consumption in Bangladesh

Seaweed has versatile uses in various biochemical raw materials, including food and foodstuff, pigments, enzymes, agar and medicine. But the value-added edible seaweed products and their acceptance has not gained in full popularity with mass consumers, but the indigenous communities of Cox's Bazar and the contiguous hill districts consume such products. As part of its efforts to popularize seaweed as well as its use in cosmetics, a fair was hosted earlier to showcase seaweed products made by local farmers and entrepreneurs. Twenty-five carts were also distributed among seaweed food entrepreneurs there. -e entrepreneurs use seaweed in different recipes such as fried crabs and faluda. Seaweed-mixed ready-to-eat foods include salad, cookies, chips, burger, cake, chanachur (fried seasoned gram) and jelly while ready-to-cook items include soup, noodles and tea. -e plant is also used as a garnish on pudding, chapati and samosa. Bangladesh should be

instrumental in popularizing seaweed food, consider seaweeds as a potential economic crop in the blue economy initiative.

Seaweed food has found its place on shelves in restaurants, especially in Cox's Bazar tourist spots. Local pharmaceuticals and cosmetic companies are also interested in using seaweed-derived products (carrageenan, alginic acid and gelatin) in their industries. A research work, conducted jointly by Chattogram University, and Food and Agriculture Organization (FAO), estimated the annual seaweed utilization in the human food, feed, manure, cosmetics and pharmaceutical industries in Bangladesh at 47,775 kilo, 11,700 kilo, 13,650 kilo and 24,375 kilo respectively. According to the research, these could potentially contribute Tk 55.87 million to the blue economy of Bangladesh in the near future. Seaweed is also in good demand among consumers in Bangladesh’s frontier neighbor Myanmar.

Chapter-3 Materials and Methods

3.1 Collection of seaweed sample

Hundred percent naturally grown sample of *Porphyra umbilicalis* were collected from two (previously) selected sampling areas: Inani rock beach and Patenga sea beach. Collected seaweed samples were transported back to the laboratory of Applied food science and Nutrition as soon as possible and stored at 4℃ until required for analyses.

3.2 Preparation of the collected sample

Collected purple laver samples of 250 gram from both Inani rock beach and Patenga sea beach were washed under running tap water to remove. Following that, each sample was shade dried at room temperature (25℃). Dried samples were ground using a grinder until the powder was homogenous. To remove any remaining residue, the powder sample was passed through a fine (2mm mash) sieve. Prior to use, the fine powder samples were stored in labeled airtight plastic containers.

3.3 Study Design

First, red seaweed samples (*P. umbilicalis*) were collected from different areas of a previously selected natural waterbody. After collecting the samples, they were thoroughly washed to remove sand, clay, and other particles. Then the cleaned samples dried and ground into powder form and stored in room temperature until analysis. The powder sample was then analyzed to determine its proximate composition (moisture, ash, crude fat, protein, crude fiber, carbohydrate, amino acid and fatty acid), mineral contents (Sodium, Potassium, Calcium, Magnesium, Iron, and Zinc), Vitamin A (carotinoids) and Vitamin E (α-Tocoferol) content.

3.4. Methods of analysis

The macronutrient components of the samples including crude protein, total carbohydrates, crude fat, amino acid, fatty acid content, crude fiber, vitamin A (carotenoids) and vitamin E (α-tocoferol) were estimated using chemical analysis on a moisture- free basis.

3.5. Proximate analysis

3.5.1. Determination of crude protein

Digestion: 0.3g sample, 4g catalyst and 5 ml H_2SO_4 was taken in a kjeldahl digestion tube. It was placed in the digestion unit and digested for at 32°C for 30 minutes. The digestion was completed when the color of the substance was pale yellow.

Distillation: After cooling the digestion tube at ro om temperature 25ml distilled water, 25 ml 35% NaOH and glass blitz were added to kjeldahl flask which containing about 10 ml 4% boric acid and 2-3 drops mixed indicator. Cooled tube and receiving solution were placed into the distillation unit. 25ml of 35% NaOH was automatically filled into the tube. The distillation process takes place for 3 minutes. The receiving solution turned green at the end of the process.

Titration: The solution collected was titrated with 0.2N HCl solution and titer value was recorded.

3.5.2 Determination of crude fat

Procedure: The dried sample remaining after moisture determination was transferred to a thimble and plugged the top of the thimble with a wad of fat free cotton. The thimble was dropped into the fat extraction tube attached to a Soxhlet flask. Approximately 75ml or more of anhydrous ether was poured into a flask. The top of the fat extraction tube was attached to the condenser. The sample was extracted for 16hrs or longer on a water bath at 800C. At the end of the extraction period, the thimble was removed from the apparatus and distilled off most of the ether by allowing it or collected in Soxhlet tube. The ether was poured off when the tube was nearly full. When the ether reached a small volume, it was poured into a small, dry beaker through a small funnel containing a plug of cotton. The flask was rinsed and filtered thoroughly, using ether. The ether was evaporated on a steam bath at low heat; it was then dried at 1000C for 1hr, cooled and weighed. The difference in the weights gave the ether soluble material present in the sample.

3.5.3 Determination of moisture content

At first weight of empty crucibles were dried and 5gm of sample was placed on it. Then the crucible was placed in an air oven (thermostatically controlled) and dried at temperature

of 105◌̊C for 24 hrs. After drying, the crucible was removed from the oven and cooled in desiccator. It was then weighed with cover glass. The crucible was again placed in the oven, dried for 30 minutes, took out of the dryer, cooled in desiccator and weighed. Drying, cooling and weighing were repeated until the two consecutive weights were same.

3.5.4 Determination of ash content

The ash content of the samples was determined by the standard AOAC method (AOAC, 2016). In this method, an empty crucible was cleaned properly and dried in a hot air oven. It was placed in desiccators and cooled then the weight was recorded. 3 gm of the sample was weighed and placed in the crucible. It was allowed to burn up to no smoke. The crucible was cooled and transferred to the muffle furnace at 550℃ for 5 hours. The process ends when formation of white ash accomplished. It was cooled at 150℃ and then placed to desiccator. When it cooled to mild warm the weight was recorded.

3.5.5 Determination of crude fiber

Crude fiber was determined according to AOAC method (2016). At first 2 gm of the sample was weighed and then taken into a beaker. Then 125ml 0f 1.25% sulfuric acid solution and 3-4 drops of n-octanol were added into the same beaker. N-octanol was using as an antifoaming agent. The beaker was boiled for 30 minutes at constant volume. After that, the sample was washed three times to remove the acid. After washing 125ml of 1.25% sodium hydroxide and 3-5 drops of antifoam were added. It was again boiled for another 30 minutes at constant volume. The mixture was filtrated and again washed the residue like before. It was washed again with 1% HCL solution in order to remove the acid. Then the residue was dried in a hot air oven at 105℃ until a constant weight was found out. It was placed in a desiccator for cooling and the weight was recorded. Finally, the residue was burned up to smoke and ignited in the muffle furnace at 550-660℃ for about 3-4 hours until that turned into white ash.

3.5.6 Estimation of total carbohydrate

The available carbohydrate content was determined by subtracting the sum of the values of moisture, ash, protein and fat from 100 (per 100gm) (AOAC, 2016). Hence it was calculated using the formula below:

3.6. Determination of Amino Acid

A small modification of Benjama and Masniyom (2011) was used to determine the amino acid composition of G. fisheri. This was accomplished by utilizing a High Performance Liquid Chromatography (HPLC LC-2050 series) system (Waters Corporation e2695, Milford, MA, USA) equipped with a degasser, auto sampler, and fluorescence detector to carry out the Water AccQ.Tag Amino acid analysis method.

Each sample was analyzed twice, using the same amino acid analysis method. A volumetric flask holding 50 mL was labeled and filled with 2 gm of each sample. To each of the conical flasks, 20 mL of 6N HCl was added. The volumetric flasks' caps were then screwed on securely, making them airtight. The hydrolysis was carried out by placing each flask in an oven preheated to 105°C for a period of 24 hours. After the hydrolysis was complete, the flasks were allowed to cool and 20 mL of water was added to each solution. After a thorough mixing, the solution was strained through Whatman filter paper. The pH was adjusted to 7:10 with 0.1N NaOH after the filtrate was collected. After that, 2 mL of each solution was filtered using a 0.2 m syringe and stored in a sample vial. In order to replicate the results, 50 L of reagent powder and 50 L of reagent diluent were pipetted from one vial (reagent + sample) to another vial (reagent + diluent) and labeled accordingly. About 350 L of AccQ-Fluor Borate Buffer was pipetted into this vial and vortexed for ten minutes. About 150 L of the solution was pipetted into the HPLC inner tube for each vial (reagent + sample).

High Performance Liquid Chromatography System (Model: Waters e2695, Waters Corporation, USA) equipped with Fluorescence detector (FLR 2475) and C8 column (150 mm) was used to analyze amino acids. These are the instrumental parameters: Maintain a flow rate of 1 mL/min through a column kept at 37˚C, with an injection volume of 10 L and a detection wavelength of 250-395 nm in size using fluorescence detection. Aqueous Buffer, Acetonitrile, and Deionized Water were used as mobile phases in the procedure.

3.7. Determination of fatty acid:

Fatty acid content was determined according to the official method of AOAC 969.33.

(a) For fats and oils- Sample was added to flask and then methanolic NaOH solution was added to the boiling chip. Condenser was attached and reflux until fat globules disappeared (usually 5-10 min).

From bulb or automatic pipet, BF solution was added through condenser and continued boiling for 2 min. 2-5 mL heptane was added through condenser and boilled min longer. After removing the heat and then condenser, ca was added to 15 mL saturated NaCl solution. Stopper flask was placed and shaked vigorously for 15 s while solution was still tepid. Additional saturated NaCl solution was added to floated heptane solution into neck of flask. Then ca was transferred 1 mL upper heptane solution into glass-stoppered test tube and small amount anhydrous Na_2SO_4 was added to remove H_2O. To recover dry esters, aqueous and heptane phases were transferred to 250 mL separator and extracted with two 50 mL portions petroleum ether (bp 30-60°C) or hexane. After that, combined extracts were extracted with 20 mL por-tions H_2O until acid-free to methyl red indicator and dried over anhydrous Na_2SO_4,, filter, and evaporate solvent under stream of N, on steam bath.

(b) For fatty acids- fatty acid was added to the flask with BF solution, and continued as in (a) with 2 min boiling under reflux.

3.8. Determination of Vitamin E as α-Tocopherol

Preparation of Samples- 2 gram to 10 gram of sample were taken into 250 ml conical flask. 50 ml of 95% ethanol, 50ml of 50% KOH and 0.25g of ascorbic acid were added in the flask. Then the flask was attached to water cool refluxing apparatus and the temperature was adjusted to give a reflux rate of about 2 drops per second. Switch of the hot plate was turned and the sample was heated by refluxing process for 30 minutes at 40°C. After that, the unit to room temperature. Finally, the solution was transferred into separating funnel and rinse the flask with 50 ml of distilled water.

Extraction- 25 ml petroleum ether was added in the solution and shacked vigorously to separate the solution into 2 layers. The upper solution (petroleum ether extract) was clearer than the lower solution (sample). The petroleum ether extracts were collected by removing the lower solution into a beaker throw waste pipe. Then, the petroleum ether extracts were kept into another new beaker. The lower solution were kept into a separating funnel again

and the extraction steps were repeated by adding 25 ml ether for another 2 times. Finally, the ether extracts were combined together and washed with water until solution was neutral to phenolphthalein. Then, the ether extract was filter washed through anyhydrous sodium sulphate and evaporated to dryness under N_2. 10 ml of methanol was added to dilute the solution and prepared to be analyzed by HPLC **(**Injection volume – 10 µl).

3.9. Determination of Vitamin A as Carotenoids

The total carotenoids (TCC) determined by the method of Kumar, Ramakritinan, & Kumaraguru (2010). Freeze dried 3 g *porphyra umbilicalis* powder was extracted With 75 ml. of hexane:acetone:ethanal (2:1:1, v/v) for hour at room temperature (RT) (24°C). The homogenate was filtered using Whatman No.1 filter paper and the supernatant collected was made up to 100 mL with extraction solvent. Next, 25 mL of water was added and shaken vigorously. Separation of the phase took place after 30 min. Two layers were observed, organic (upper layer) and aqueous (lower layer). The absorbance of the organic layer was measured at 470 nm and the TCC was calculated using the following formula (de Carvalho et al., 2012):

Carotenoid content (ug g") = [A x v (mL) x 10*] / A" x w (g), where A=absorbance; v = total Extract volume; w = sample weight; A'™ = 2600 (B-carotene extinction coelficient in hexane).

3.10. Mineral analysis

Wet digestion was used to extract minerals from the organic food matrix in accordance with the AOAC ICPOES Ashing procedure. Utilizing a biochemical analyzer (Humalyzer 3000®), it was possible to detect the mineral contents of the digested components (sodium, potassium, magnesium, calcium, phosphorus, iron, and zinc). For the biochemical assay, a commercially available biochemical kit (Randox) was employed.

Digestion:

One (01) g of dry sample was weighted in a conical flask. For dried samples, 7.5 mL conc. HNO_3, and 2.5mL conc. $HClO_4$ in the ratio of 2:1 was prepared. For wet sample, 5 mL

HNO_3 and 1 mL $HClO_4$ was added (HNO_3: $HClO_4$ = 5:1). The flask was then heated at 200W for 1-2 hours until full digestion was achieved. It was chilled to room temperature after digestion. The digested samples were then put into a 100 mL volumetric flask, diluted with Deionized water to the 100 mark, and thoroughly mixed. The solution was then transferred to an Eppendorf tube for mineral measurement after being filtered via Whatman filter paper No. 1.

3.10.1 Determination of Sodium (Na)

Magnesium and Uranyl acetate are used to precipitate sodium as a triple salt. In an acidic media, excess uranyl ions react with ferrocyanide to produce a brownish color.The amount of sodium present in the sample has an inverse relationship with the color intensity that results.

Step 1: Precipitation

Table 1: Sample and Standard preparation

	Pipette into cuvette	
	Blank	**Standard**
Precipitating Reagent(L1)	1.0 ml	1.0ml
Sodium Standard	20 µl	-
Sample	-	20 µl

Mix well and let stand at R.T. for 5 minutes. With shaking well intermittently. Centrifuge at 2500 to 3000 RPM to obtain a clear supernatant.

Step 2: Color Development

Table 2: Sodium (Na) Determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Acid Reagent (L2)	1.0 ml	1.0 ml	1.0 ml
Supernatant from step 1.	-	20µl	20µl
Precipitating Reagent(L1)	20µl	-	-
Colour Reagent (L3)	100µl	100µl	100µl

3.10.2 Determination of Calcium (Ca++)

Principle: Calcium ions form a violet complex with O-Cresolphthalein complexone in an alkaline medium.

Table 3: Calcium (Ca++) determination

	Pipette into cuvette		
	Reagent blank SO	**Standard SI**	**Sample**
Sample	-	-	25µl
Distilled water	25µl	-	-
Standard	-	25µl	-
Working reagent	1.0 ml	1.0 ml	1.0 ml

3.10.3 Determination of magnesium (Mg)

Principle: The technique is based on the particular binding of magnesium and calmagite, a metallochromic indicator, at alkaline pH, which causes a shift in the complex's absorption wavelength. The amount of magnesium in the sample directly affects how intense the chromophore is.

Table 4: Magnesium (Mg) determination

	Pipette into cuvette		
	Blank	**CAL. standard**	**Sample**
Sample	-	-	10µl
CAL. standard	-	10µl	-
R1. reagent	1.0 ml	1.0 ml	1.0 ml

3.10.4 Determination of Potassium (K)

Principle: A fine turbidity of potassium tetraphenyl boron is created when potassium and sodium tetraphenyl boron combine. The amount of turbidity is inversely related to the amount of potassium present in the sample.

Table 5: Potassium (K+) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	0.02 ml
Deionized water	0.02 ml	-	-
Standard	-	0.02 ml	-
K+ reagent	1.0 ml	1.0 ml	1.0 ml

3.10.5 Determination of Iron (Fe)

Principle: In a mildly acidic media, the iron is dissociated from the transferring-iron complex. Ascorbic acid is used to convert liberated iron into the bivalent state. With FerroZine, ferrous ions produce a colorful complex. The amount of iron present in the sample directly correlates with the intensity of the color that results.

Table 6: Iron (Fe) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	200µl
Standard	-	200µl	-
Reagent	1.0 ml	1.0 ml	1.0 ml

3.10.6 Determination of Zinc (Zn)

Principle: Nitro-PAPS and zinc combine to generate a purple complex in an alkaline media. The intensity of the complex produced is inversely correlated with the concentration of zinc in the sample.

Table 7: Zinc (Zn) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Working reagent	1.0 ml	1.0 ml	1.0 ml
Distilled water	50µl	-	-
Zinc standard	-	50µl	-
Sample	-	-	50µl

Chapter-4: Results

After conducting all the biochemical analysis of the dried seaweed samples, collected data were determined and stored in Microsoft Excel 2013 spread sheet to evaluate statistical analysis. All samples were in three replicates. Descriptive statistics (mean and standard deviation) were done for proximate composition.

4.1. Proximate composition of red seaweed (*P. umbilicalis*)

The proximate composition of red seaweed (*P. umbilicalis*) was assessed in the present study and showed in Figure 4.1. The percentage of carbohydrate content was found to be almost half of the total nutritional composition (49.32± 0.67%). 21.60±0.44% protein, 16.42±0.47% ash, 6.06±0.50% moisture and 5.44±0.01% crude fiber was found in the collected sample. Very low percentage of lipid content (0.50±0.25%) was found in the sample.

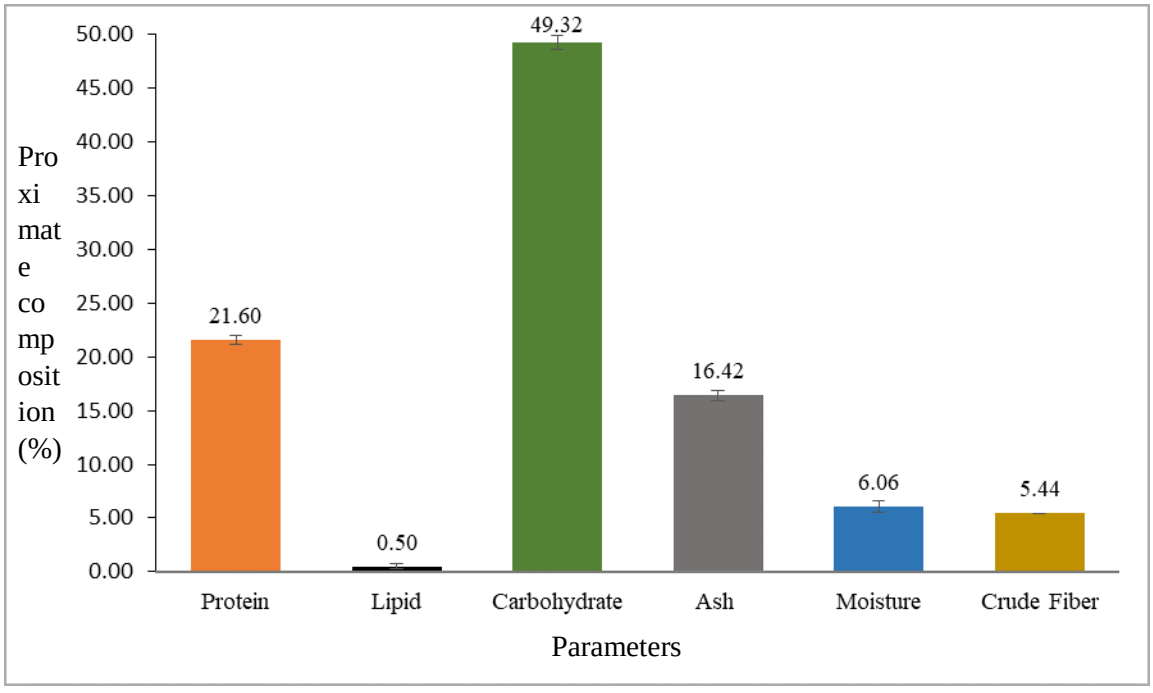

Figure 4.1. Proximate composition (protein, lipid, carbohydrate, ash, moisture and crude fiber) of *P. umbilicalis* collected from Inani rock beach and Patenga sea beach

4.2. Amino acid composition of *Porphyra umbilicalis*

The amino acid composition of *P. umbilicalis* (100 g protein)$^{-1}$ is presented in Figure 4.2. The protein of *P. umbilicalis* contained all the essential amino acids (EAAs) at different proportion (excluding tryptophan, which was eliminated after acid hydrolysis). For NEAAs, tyrosine constituted a considerable amount in *P. umbilicalis*. Other NEAAs such as cysteine, serine, tyrosine and arginine were also found in considerable amount. In comparison with other AAs in the current study, valine had the highest content (230.5) and lysine had the lowest content (87.5).

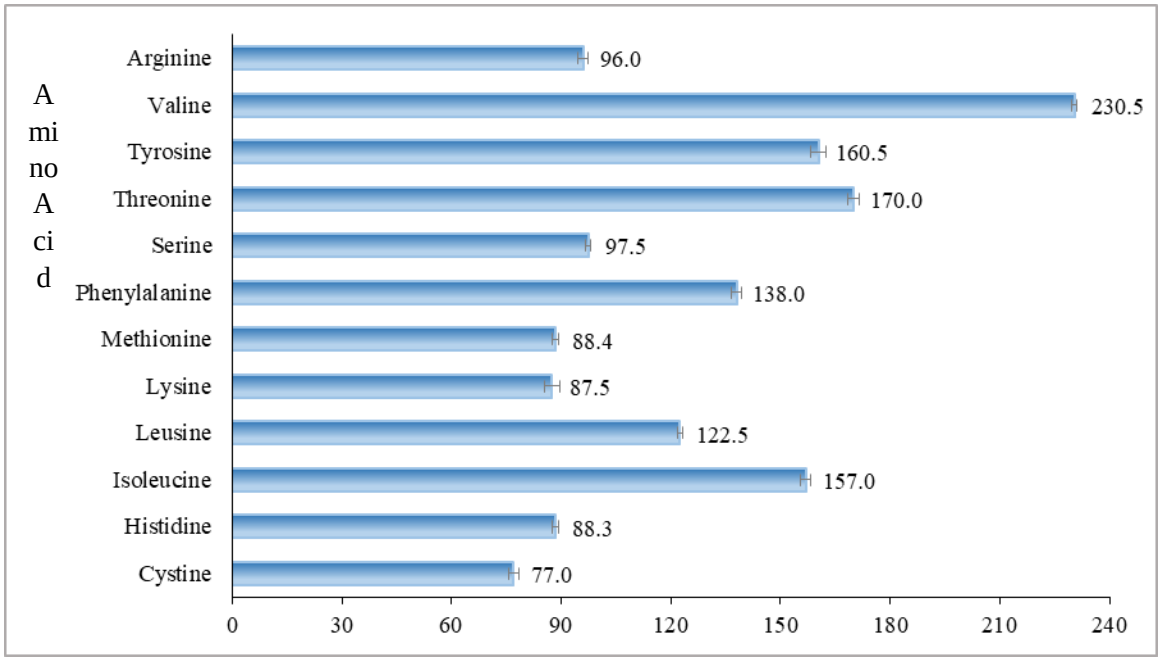

Figure 4.2. Amino acid composition (both essential and non-essential) of *P. umbilicalis*

4.3. Fatty acid composition

Fatty acid composition of the collected samples were also determined in this investigation (Table4.3). The percentages of different fatty acid groups based on their carbon chain and double bonds are presented in Figure 4.3. It was found that, almost half of the fatty acid composition is consist of PUFA (Poly unsaturated fatty acids) followed by SFA (Saturated fatty acids) and MUFA (Monounsaturated fatty acids). 55.96% of different groups of polyunsaturated fatty acids were dominant in this red algae. A moderate amount of saturated fatty acids were also present (34.75 %) with minimum quantity of monounsaturated fatty acids (9.29%). Thus, the distribution of fatty acids in this red algae appears to be polyunsaturated> saturated> monounsaturated

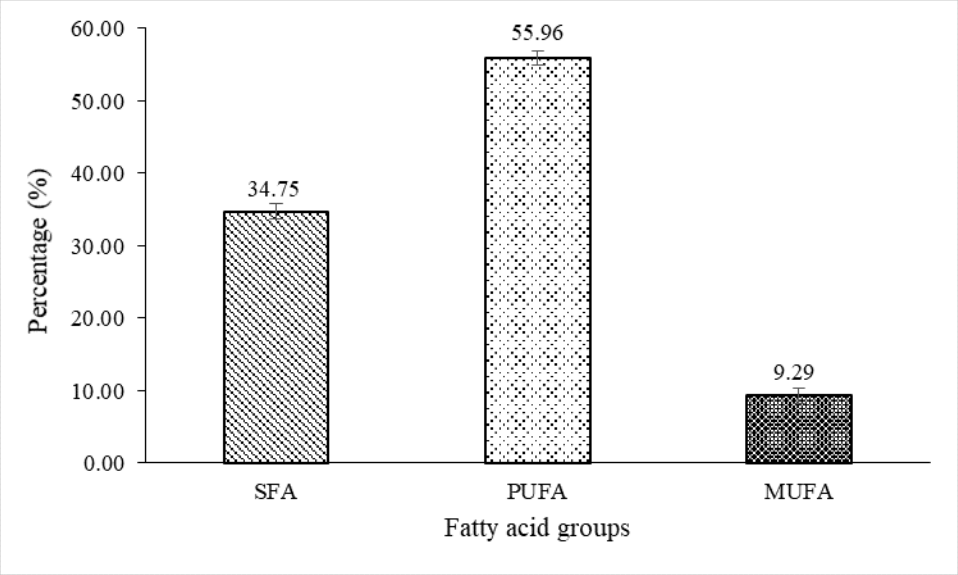

Figure 4.3: Percentages of different fatty acid groups (SFA, PUFA, and MUFA)

Table 4.3. Fatty acid composition of *P. umbilicalis*

Sl. No.	Fatty Acid	Value	SE
1	Methyl Octanoate (111-11-5)	0.2704554	0.256074
2	Methyl Decanoate(110-42-9)	0.2279307	0.217594
3	Methyl Laurate(111-82-0)	0.1535609	0.032054
4	Methyl Tridecanoate(1731-88-0)	0.2130783	0.029935
5	Methyl Myristate(124-10-7)	0.3240343	0.162025
6	Methyl Palmitpleate(1120-25-8)	5.5633434	4.238196
7	Methyl palmitate(112-39-0)	1.5303554	1.125333
8	Methyl Linoleate (112-63-0)	31.972251	30.35216
9	Methyl Oleate (112-62-9)	1.4122941	0.96677
10	Methyl LInolenate (301-00-8)	8.4250747	6.965772
11	Methyl Stearate (112-61-8)	1.13904	0.693515
12	Methyl Arachidate (1120-28-1)	16.198549	14.86666
13	Methyl Arachidonate (2566-89-4)	0.4081416	0.361401
14	Methyl Eicosapaennoate (2734-47-6)	2.8448305	1.650917
15	Methyl 11-14-17-Eicosatrienoate(55682	2.0921741	0.742982
16	Methyl Heptadecanoate (1731-92-6)	0.5900968	0.422459
17	Methyl Heneicosanoate (6064-90-0)	4.2196803	3.23273
18	Methyl Docosahexanoate (2566-90-7)	0.6355823	0.190058
19	Methyl Docosapentaenoate (2566-90-7)	0.0440977	0.036907
20	Methyl Eirocate (1120-34-9)	13.10369	8.72945
21	Methyl 11-Eicosapentaenoate (2390-09-2)	1.5906891	1.365974
22	Methyl Hehenate (929-77-1)	0.215556	7.078576
23	Methyl Tricosanoate (2433-97-8)	-	-
24	Methyl Nervonate (2733-88-2)	0.1634696	0.160548
25	Methyl Lignocerate (2442-49-1)	-	-

*SE= Standard Error

4.4. Vitamin A (carotinoids) and Vitamin E (α-Tocoferol) content

The vitamin content of red algae is shown in Table 4.4. A very low concentration of vitamin A and vitamin E was found from the collected samples. An average value of 2.415±0.043 mg/kg of Vit. E (α-Tocopherol) and 1.823±0.024 µg/L of Vit. A (carotenoids) were determined from the collected red seaweed samples (*P. umbilicalis*).

Table 4.4 Vitamin content of red algae (*P. umbilicalis*).

Parameters	Value	SE
α-Tocopherol	0.2415 mg/kg	0.043
Carotenoids	1.823 µg/L	0.024

*SE= Standard Error

4.5. Mineral Analysis

Mineral composition of *P. umbilicalis* are presented in Table 4.6. Four macro molecules and two micro molecules were determined in this study. Almost all the sample contained highest amount of potassium (K) and sodium (Na) than other minerals. Higher amount of Na, K, Mg, Ca, content were found from 100g of seaweed sample.

The concentration of micro molecules such as Iron and Zinc were found to be lower (253.6 mg and 6 mg respectively) in respect with the macro molecules (potassium- 1428 mg, calcium- 313.8 gm, 714.6 mg and 1256.7 mg).

Table 4.5 Mineral composition (mg/100g dry basis)

Minerals		Composition (mg/100g)
Macro minerals	Potassium (K)	1428
	Calcium (Ca)	313.8
	Megnesium (Mg)	714.6
	Sodium (Na)	1256.7
Micro minerals	Iron (Fe)	253.6
	Zinc (Zn)	6.0

Chapter-5: Discussion

The discovery of metabolites and biological activity from macroalgae has dramatically increased over the past three decades. There are many advanced current research techniques for creating chemical compounds, yet many naturally occurring bioactive molecules still exist in nature's womb and are a mystery. Scientists are still searching for additional physiologically active compounds. Pharmaceutical companies and researchers are increasingly paying increased attention to the bioactive compounds found in seaweeds as they work to develop new drugs. Seaweeds are receiving the bulk of scientific attention as a result of their bioactive phenomena and several beneficial features, including those that are anti-viral, anti-tumor, anti-inflammatory, and anti-lipedemic. This study primarily discusses the nutritional value and other characteristics of red seaweed (*P. umbilicalis*).

5.1. Proximate composition of red seaweed (*P. umbilicalis*)

The proximate composition of red seaweed (*P. umbilicalis*) was assessed in the present study and showed in Figure 4.1. The percentage of carbohydrate content was found to be almost half of the total nutritional composition (49.32± 0.67%). 21.60±0.44% protein, 16.42±0.47% ash, 6.06±0.50% moisture and 5.44±0.01% crude fiber was found in the collected sample. Very low percentage of lipid content (0.50±0.25%) was found in the sample. The amount of light (for photosynthesis) and nutrients in the water, as well as the water's temperature, have a significant impact on the growth rate and nutritional makeup of red seaweeds. According to the location and time of year, these variables change (Hagen et al., 2004).

5.1.1. Protein

In the red seaweeds, nori contains significantly higher protein levels (Muhammad et al., 2021). Figure 4.1 shows that, protein content of *P. umbilicalis* was found to be 21.60±0.44% in this study. According to previous studies, the amount of protein found in red algae varies with an average range of 18.8 ± 7 g/100 g (Vega et al., 2020). In the present study, red seaweeds contained higher protein levels (~19–21%) similar to those observed by Fernández-Segovia et al. (2018). In general, red algae have a higher protein content than green and brown algae (Fleurence et al., 2012; Courtois, 2008; Fleurence, 1999). The

literature consulted for this study indicates that the protein content of red algae ranges from 5.2% to 40% (dry weight). The high protein content of nori algae (*P. umbilicalis*), which is comparable to the protein content of soybeans, according to the authors of one study, stands out. However, compared to proteins of animal origin, the digestibility of algae protein is only moderate; this may be because algae have a high concentration of dietary fiber, which restricts the availability of digestive and proteolytic enzymes.

Phycobiliproteins, which can make up to 50% of a red seaweed species' total protein content, are pigment proteins. Red seaweed species have high protein levels that are equivalent to terrestrial plant species that are important sources of protein (2013). For instance, protein levels of red seaweed species like *Porphyra tenera* (nori) and *Palmaria palmata* (dulse) have been compared to those of soybeans (2018). Additionally, there are observable differences in the protein content of red seaweeds depending on the species, seasons, and environmental factors (Muhammad et al., 2021).

5.1.2. Lipid

According to reported literature, most seaweeds contained lipid less than 4% DW (McDermid and Stuercke, 2003). But some seaweeds had high level of crude lipid, such as *Dictyota acutiloba* (16.1% DW) and *D. sandvicenis* (20.2 % DW) (McDermid and Stercke, 2003). The results of the previous findings represent that, the fat content in red algae is fairly low, with an average of 1.5 g/100 g, a minimum value is 0.2 g/100 g (in the algae *P. umbilicalis*), and a maximum of 6.2 g/100 g (in *Laurencia filiformis*).

From this research, very low percentage of lipid content (0.50±0.25%) was found in the collected sample. Cofrades et al. (2010) also reported protein (< 39%), fat (<1.5%) and ash (12%) contents for nori almost similar to levels reported in this study. In contrast, Taboada et al. (2013) observed that nori had a greater fat content (2.8%) that was made up of 43.2% saturated fatty acids, 26.6% monounsaturated fatty acids, and 26.9% polyunsaturated fatty acids (23.9% w3 and 2.6% w6). The methods used for the lipid analysis may have been the cause of the discrepancy. In this investigation, the Soxhlet extraction method (AOAC, 2000) with a 2:1 combination of chloroform and methanol (Bligh and Dryer, 1959) was used to modify the detection of lipid content. The employment of various procedures or regional and seasonal conditions could be to blame for this minimal amount of extracts

(Haroon et al., 2000). All algae, on average, have low lipid contents, but the fat they do have is of excellent nutritional quality (Kumar et al., 2008).

5.1.3. Carbohydrate, ash and crude fiber content

Red algae's carbohydrate content was high overall but varied greatly among the experimental samples. The lowest concentration of carbohydrates was found in the Hypnea genus, which had an average of 15.1 g/100 g; much greater concentrations were found in the Gracilaria and Pyropia genera, with 47.9 and 49.5 g/100 g, respectively. It was discovered that the percentage of carbohydrates made up about half of the entire nutritional makeup (49.320.67%). The primary D-glucan found in red algae is amylopectin, also known as floridean starch. Floridoside is a valuable store of soluble carbon that cells can utilize. It functions in aqueous solutions as an osmoregulator and is in charge of the algal cell wall's resilience to changes in the medium's salinity. The immune system may benefit from an extract from the red algae Mastocarpus stellatus, according to a study by Courtois et al. (Courtois, 2008). Contrary to what happens with protein content, the concentration of extract rises in the summer and falls in the winter (Hagen et al., 2004). The red algal cell wall, according to Lee et al. (Lee et al., 2017), has a large quantity of agar, a polysaccharide made of D- and L-galactose, along with some sulfated side chains, methoxy groups, and other components. Agar is a phycocolloid that is used in food, medicine, cosmetics, and other products.

Table 4.1 demonstrates the high dietary fiber content of red algae (5.440.01%). According to Guérin-Deremaux et al. (2011), the amounts of soluble dietary fiber are good for your health since they lower blood cholesterol and postprandial glucose levels while also making you feel fuller. The average value of 16.420.47%, which is significantly greater than that of terrestrial vegetables, is indicated for the ash content of the red algae samples in Table 4.1. This is related to mineral content and varies by geographic region and season (Sánchez-Machado et al., 2004; Siddique et al., 2013).

5.2. Amino acid profile of protein (*P. umbilicalis*)

Analyzing the amino acid profile of the processed *P. umbilicalis* sample, the researchers found that it was quite comparable to what had been described before. Table 4.2 shows, 12 amino acids (Arginine, valine, tyrosine, threonine, serine, phenylalanine, methionine, lysine, leucine, isoleucine, histidine and cysteine) obtained from control acid oxidation, acid hydrolysis and alkaline hydrolysis by using HPLC. The amino acids valine, threonine, isoleucine, and phenylalanine had the highest values. Leucine, valine, and methionine, three important amino acids, are present in red algae in levels equivalent to those in egg albumin, claims one study (Astorga-Espaa et al., 2016). Red algae include quantities of other amino acids such isoleucine and threonine that are comparable to those found in plants in the legume family (Vega et al., 2020).

Red algae has between 14% and 19% essential amino acid content, compared to green algae's 26% to 32% and brown algae's total of 22% to 44% (Fleurence, 1999). However, according to Belghit et al. (Belghit et al., 2017), the concentration of some amino acids, such as glutamate, ornithine, citrulline, serine, and glycine, are significantly higher in red algae than brown and green algae. The bioavailability of the amino acids that comes from the digestion of red algae protein, which can be affected by various antinutritional agents such as polyphenols, polysaccharides, and glycoproteins (Vega et al., 2020).

5.3. Fatty acid composition

All algae, in general, have modest lipid concentrations, but the fat they do have is of excellent nutritional quality (Kumar et al., 2008). Some red algae, such G. lemaneiformis and Ceramium diaphanum, have monounsaturated fatty acid concentrations of 30%, but the majority have concentrations of about 10% to 20%, which is almost compatible with our current findings. All of the red algae examined in the experiments included oleic acid, although the amounts varied greatly, with larger concentrations being identified in the genus Gracilaria. Gelidiella acerosa, an Indian species, had a minimum value of 0.09%, and G. lemaneiformis B., a Chinese species, had a highest value of 31.1%. The greatest concentrations of linolenic and linoleic essential fatty acids in red algae are 22% and 11%, respectively. The average amount of arachidonic acid found in different kinds of red algae is 8.6%.

After examining red, brown, and green algae, Belghit et al. (2017) came to the conclusion that red and green algae contain higher concentrations of monounsaturated and polyunsaturated fatty acids than brown algae, while red algae of the Porphyra genus contained higher concentrations of fatty acids with 20 and 22 carbon atoms and oxylipines. This latter substance was created from free fatty acids, primarily linoleic and linolenic, and serves as a secondary metabolite and a component of the plant's immune system.

Fish and algae include the long-chain polyunsaturated EPA and DHA fatty acids, which belong to the omega-3 family. Based on the data on fatty acids and the omega-6/omega-3 ratios of red algae, this type of fatty material is of excellent quality and is healthy. In this study, the distribution of fatty acids in the chosen red algae (P. umbilicalis) appears to be polyunsaturated> saturated> monounsaturated.

5.4. Vitamin A (Carotenoids) and Vitamin E (α-Tocoferol) content of *P. umbilicalis*

Table 4.4 displays the vitamin content of red algae; however, this study only discovered scant evidence of vitamin content. In other trials, vitamin levels were not measured at all, and when they were, the numbers given differed greatly. In comparison to earlier research, a very low concentration of vitamins A and E was discovered overall. Rajapakse and Kim (2011) assert that red and brown algae are abundant in vitamins C and A. B vitamins are abundant in porphyra red algae (Kumar et al., 2008). Low quantities of vitamin B12, which are mostly produced by bacteria and organisms of animal origin, have been found in some algae. Red algae have also been shown to contain a substance known as pseudovitamin B12, which is not biologically active in humans (Saini and Keum, 2018).

5.5. Mineral content

According to seaweed species, oceanic residence period, geographic location of harvest, wave exposure, seasonal, yearly, environmental, and physiological parameters, as well as processing type and mineralization method, mineral content has been demonstrated to vary (Mabeau and Fleurence, 1993).

Table 4.5 provides a list of the mineral concentrations present in P. umbilicalis. Due of their contact with seawater, red algae have a very high sodium content—higher than plants

grown on land. In P. umbilicalis, an average of 1256.7 mg/100 g of Na was discovered. Both the potassium concentration (1428 mg/100 g) and the Na/K ratio (0.88) are extremely high; ideally, they should both be less than or equal to 1 (or lower), as high Na/K ratios (more than 2.5) are linked to cardiovascular disease and hypertension (Oliveira et al., 2019).

Another potential health benefit of red algae is its high magnesium content (714.6 mg/100 g), which is necessary for the synthesis of cell proteins and therefore aids in maintaining the structure of the muscle. Magnesium concentrations in most red algae are higher than those in foods like sunflower seeds, almonds, hazelnuts, wheat germ, and soybeans, which are all regarded as being high in magnesium (Gil, 2010). Red algae had a fairly high average zinc content of 6.0 mg/100 g. Since this mineral affects growth and is present in high quantities in meat, eggs, and fish, it is crucial for children to consume these foods (Ruz and Pérez, 2016; Gil, 2010).

Chapter-6: Conclusions

Relatively high levels of macro elements, essential amino acids, fatty acids, and soluble and insoluble dietary fibers are also present in red seaweed (*P. umbilicalis*), as are significant levels of ash, substantial protein, and dietary fiber. As a result, this seaweed can help meet the nutritional needs of both humans and animals. The physicochemical characteristics of *P. umbilicalis* species and their nutritional contents point to the possibility of using them as useful components in the food sector. Additionally, because they can lower blood cholesterol levels, obesity, and the risk of coronary heart disease, their consumption is good for your health. To give additional information for a safer and more flexible use of these seaweeds, more research on vitamins, non-starch polysaccharide compounds, and hazardous substances is required.

Chapter 7: Recommendations and Future perspectives

One of the key elements of all ecosystems is the seaweeds and sea grasses. As a result of their high productivity and crucial significance as breeding grounds for numerous commercially significant species, the studies based on them have emerged as the most crucial component. Seaweeds are utilized as dietary supplements and for medical purposes. The chemical properties of this macroalgae can be used to create new technologies like UV-sunscreens and natural anti-foulants, as well as to solve many human problems like severe diseases. The iron and other elements found in seaweeds are abundant and essential for maintaining regular bodily functions. Seaweeds will be beneficial in a variety of ways in the future, which will be good for human wellbeing. Many severe diseases, including the AIDS virus, malaria, and the herpes simplex virus, are treated with seaweeds. In the future, seaweeds might be employed as immune modulators, antiviral, antibacterial, anti-inflammatory, and antitumor agents.

Thus, regarding this area, some future research attempts may include the followings:

i. More investigation is needed to identify new useful components in Phorphyra umbilicalis that can be incorporated into food products.
ii. Additional fields of applications (such as nutraceuticals, treatments, cosmetics, and even animal feed) should be explored by using the biological components extracted from seaweed

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Appendices

Photo gallery

Figure: Weighing sample for proximate analysis.

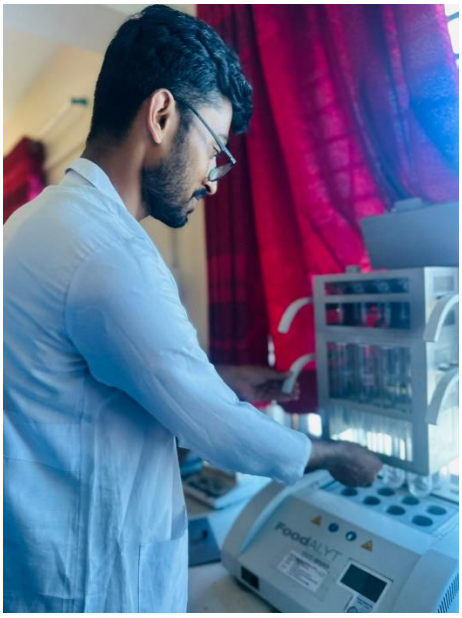
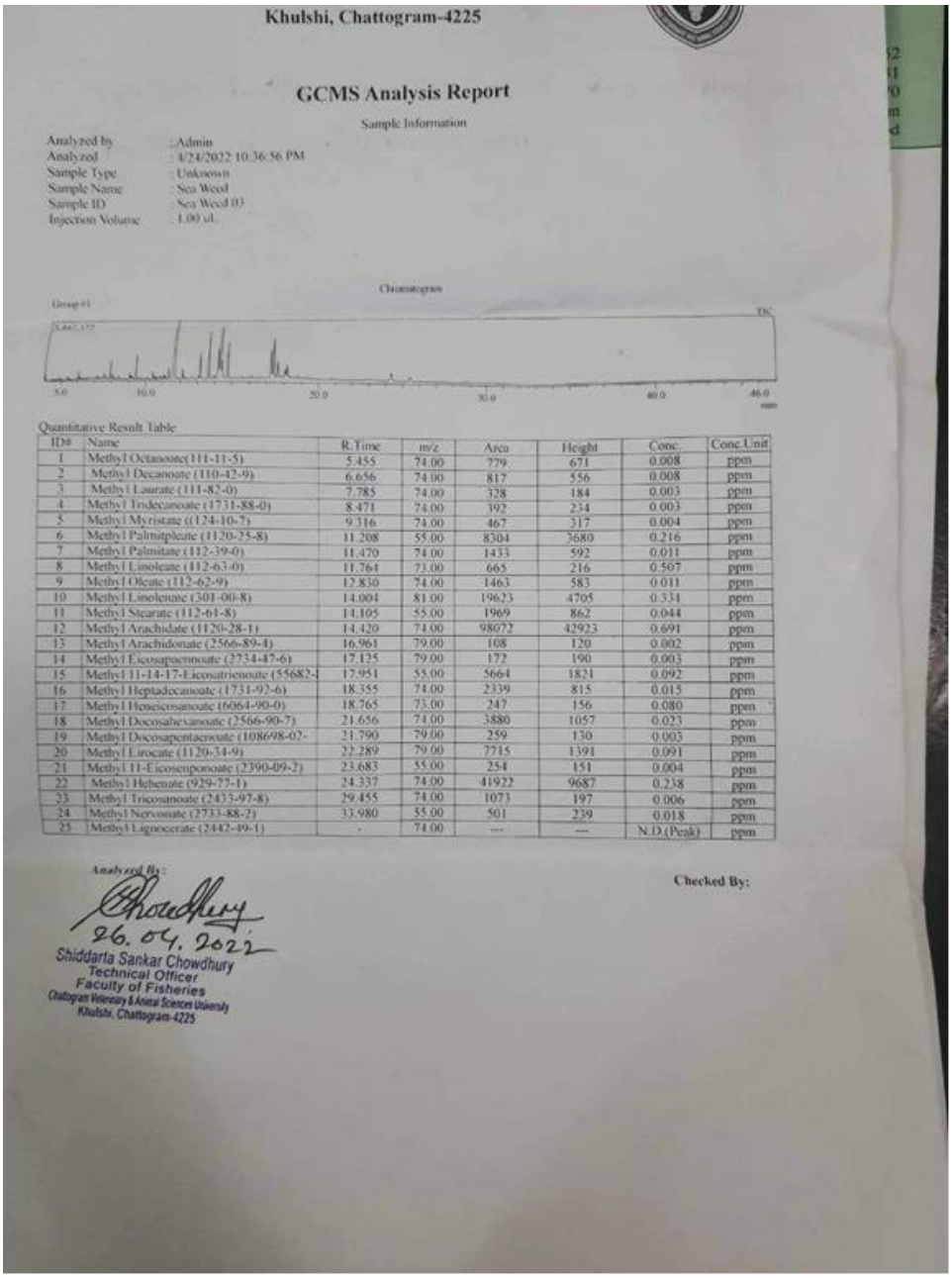


Figure: Protein determination by KJELDAHL method



Khulshi, Chattogram-4225

GCMS Analysis Report

Sample Information

Analyzed by : Admin
Analyzed : 4/24/2022 10:36:56 PM
Sample Type : Unknown
Sample Name : Sea Weed
Sample ID : Sea Weed 03
Injection Volume : 1.00 uL

Chromatogram

Group #1 TIC

5.0 10.0 20.0 30.0 40.0 46.0 min

Quantitative Result Table

ID#	Name	R.Time	m/z	Area	Height	Conc.	Conc.Unit
1	Methyl Octanoate(111-11-5)	5.455	74.00	779	671	0.008	ppm
2	Methyl Decanoate (110-42-9)	6.656	74.00	817	556	0.008	ppm
3	Methyl Laurate (111-82-0)	7.785	74.00	328	184	0.003	ppm
4	Methyl Tridecanoate (1731-88-0)	8.471	74.00	392	234	0.003	ppm
5	Methyl Myristate ((124-10-7)	9.316	74.00	467	317	0.004	ppm
6	Methyl Palmitplcate (1120-25-8)	11.208	55.00	8304	3680	0.216	ppm
7	Methyl Palmitate (112-39-0)	11.470	74.00	1433	592	0.011	ppm
8	Methyl Linoleate (112-63-0)	11.764	73.00	665	216	0.507	ppm
9	Methyl Oleate (112-62-9)	12.830	74.00	1463	583	0.011	ppm
10	Methyl Linolenate (301-00-8)	14.004	81.00	19623	4705	0.331	ppm
11	Methyl Stearate (112-61-8)	14.105	55.00	1969	862	0.044	ppm
12	Methyl Arachidate (1120-28-1)	14.420	74.00	98072	42923	0.691	ppm
13	Methyl Arachidonate (2566-89-4)	16.961	79.00	108	120	0.002	ppm
14	Methyl Eicosapaenoate (2734-47-6)	17.125	79.00	172	190	0.003	ppm
15	Methyl 11-14-17-Eicosatrienoate (55682-1	17.951	55.00	5664	1821	0.092	ppm
16	Methyl Heptadecanoate (1731-92-6)	18.355	74.00	2339	815	0.015	ppm
17	Methyl Heneicosanoate (6064-90-0)	18.765	73.00	247	156	0.080	ppm
18	Methyl Docosahexanoate (2566-90-7)	21.656	74.00	3880	1057	0.023	ppm
19	Methyl Docosapentaenoate (108698-02-	21.790	79.00	259	130	0.003	ppm
20	Methyl Erucate (1120-34-9)	22.289	79.00	7715	1391	0.091	ppm
21	Methyl 11-Eicosenponoate (2390-09-2)	23.683	55.00	254	151	0.004	ppm
22	Methyl Hebenate (929-77-1)	24.337	74.00	41922	9687	0.238	ppm
23	Methyl Tricosanoate (2433-97-8)	29.455	74.00	1073	197	0.006	ppm
24	Methyl Nervonate (2733-88-2)	33.980	55.00	501	239	0.018	ppm
25	Methyl Lignocerate (2442-49-1)	-	74.00	---	---	N.D.(Peak)	ppm

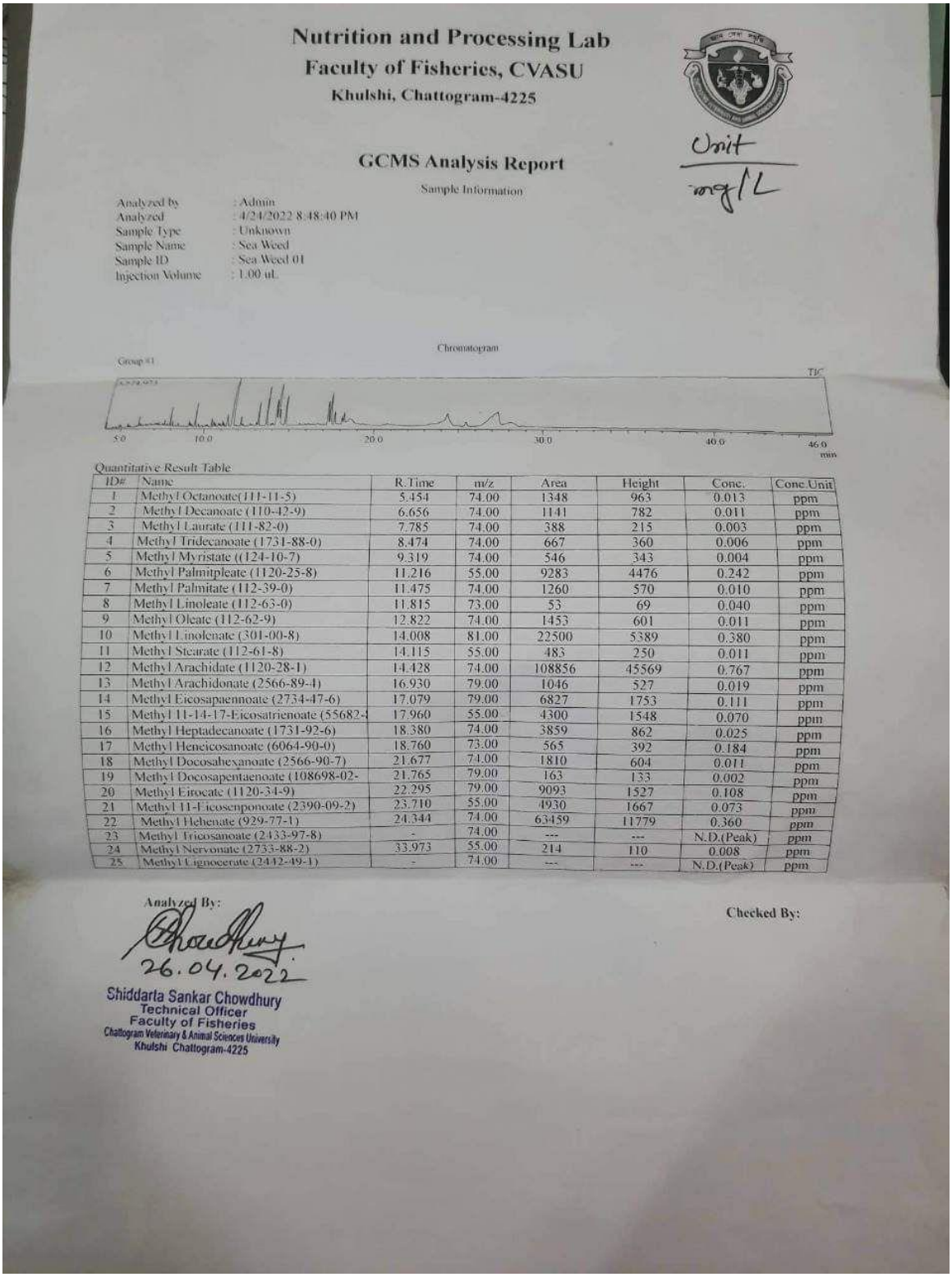
Analyzed By:

26.04.2022

Shiddarta Sankar Chowdhury
Technical Officer
Faculty of Fisheries
Chattogram Veterinary & Animal Sciences University
Khulshi, Chattogram-4225.

Checked By:

Figure: Determination of fatty acid by GCMS sample-3



Nutrition and Processing Lab
Faculty of Fisheries, CVASU
Khulshi, Chattogram-4225

Unit
mg/L

GCMS Analysis Report

Sample Information

Analyzed by : Admin
Analyzed : 4/24/2022 8:48:40 PM
Sample Type : Unknown
Sample Name : Sea Weed
Sample ID : Sea Weed 01
Injection Volume : 1.00 uL

Chromatogram

Group #1

TIC

5.0 10.0 20.0 30.0 40.0 46.0 min

Quantitative Result Table

ID#	Name	R.Time	m/z	Area	Height	Conc.	Conc.Unit
1	Methyl Octanoate(111-11-5)	5.454	74.00	1348	963	0.013	ppm
2	Methyl Decanoate (110-42-9)	6.656	74.00	1141	782	0.011	ppm
3	Methyl Laurate (111-82-0)	7.785	74.00	388	215	0.003	ppm
4	Methyl Tridecanoate (1731-88-0)	8.474	74.00	667	360	0.006	ppm
5	Methyl Myristate ((124-10-7)	9.319	74.00	546	343	0.004	ppm
6	Methyl Palmitpleate (1120-25-8)	11.216	55.00	9283	4476	0.242	ppm
7	Methyl Palmitate (112-39-0)	11.475	74.00	1260	570	0.010	ppm
8	Methyl Linoleate (112-63-0)	11.815	73.00	53	69	0.040	ppm
9	Methyl Oleate (112-62-9)	12.822	74.00	1453	601	0.011	ppm
10	Methyl Linolenate (301-00-8)	14.008	81.00	22500	5389	0.380	ppm
11	Methyl Stearate (112-61-8)	14.115	55.00	483	250	0.011	ppm
12	Methyl Arachidate (1120-28-1)	14.428	74.00	108856	45569	0.767	ppm
13	Methyl Arachidonate (2566-89-4)	16.930	79.00	1046	527	0.019	ppm
14	Methyl Eicosapaennoate (2734-47-6)	17.079	79.00	6827	1753	0.111	ppm
15	Methyl 11-14-17-Eicosatrienoate (55682-4	17.960	55.00	4300	1548	0.070	ppm
16	Methyl Heptadecanoate (1731-92-6)	18.380	74.00	3859	862	0.025	ppm
17	Methyl Heneicosanoate (6064-90-0)	18.760	73.00	565	392	0.184	ppm
18	Methyl Docosahexanoate (2566-90-7)	21.677	74.00	1810	604	0.011	ppm
19	Methyl Docosapentaenoate (108698-02-	21.765	79.00	163	133	0.002	ppm
20	Methyl Eirocate (1120-34-9)	22.295	79.00	9093	1527	0.108	ppm
21	Methyl 11-Eicosenponoate (2390-09-2)	23.710	55.00	4930	1667	0.073	ppm
22	Methyl Hehenate (929-77-1)	24.344	74.00	63459	11779	0.360	ppm
23	Methyl Tricosanoate (2433-97-8)	-	74.00	---	---	N.D.(Peak)	ppm
24	Methyl Nervonate (2733-88-2)	33.973	55.00	214	110	0.008	ppm
25	Methyl Lignocerate (2442-49-1)	-	74.00	---	---	N.D.(Peak)	ppm

Analyzed By:

26.04.2022

Shiddarta Sankar Chowdhury
Technical Officer
Faculty of Fisheries
Chattogram Veterinary & Animal Sciences University
Khulshi Chattogram-4225

Checked By:

Figure: Determination of fatty acid by GCMS sample-1

Brief Biography

Md Tarekul Islam is the 1st son of Nurul Hoque and Shahinur Akter was born in 3rd May, 1998 in Chattogram, Bangladesh. He has achieved Secondary School Certificate from Raipur Union Multilateral High School and Higher Secondary Certificate from Bangladesh Marine Academy College. He has also achieved B.Sc (Hons) in Food Science and Technology. He is now a candidate of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition at Chattogram Veterinary and Animal Sciences University.

Now he has been working in a Saudi Government Project which is monitoring by Ministry of Municipal Rural affairs and Housing. The Ministry has prepared a guide (a health education program for workers in food and public health facilities), in their capacity as (workers / supervisors) who are the common relevant in incidents of food poisoning or disease transmission. This program is implemented in private centers and institutes or entities specialized in this field. The project is specialized in the field of environmental health education and awareness and its mission is to prepare, implement the scientific program to health educating and qualifying workers in food and public health establishments to conduct exam for them in one of its branches affiliated with the Exam and Evaluation Center, which are located in all regions of the Kingdom.