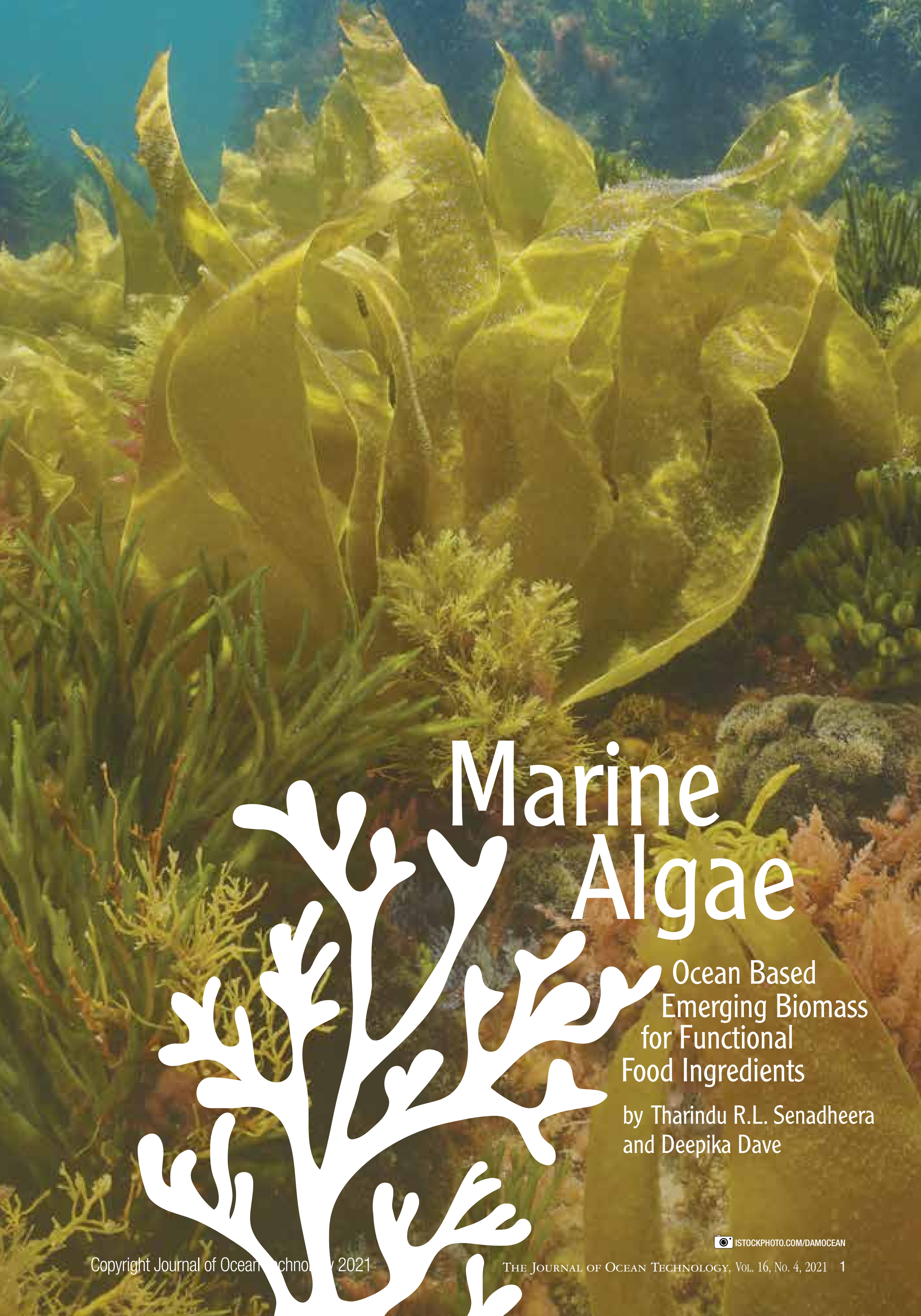

Marine Algae

Ocean Based Emerging Biomass for Functional Food Ingredients

by Tharindu R.L. Senadheera and Deepika Dave

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Marine algae are one of the richest sources of bioactive products in the marine environment and represent over 90% of all marine plants. These photosynthetic organisms do not have true roots or stems and consist of a large number of species that originate from unicellular microscopic organisms (microalgae) or multicellular organisms of varying size (macroalgae). Marine macroalgae are commonly known as seaweeds and according to their pigmentation they are classified into three distinguishable groups as brown (Pheophyta), red (Rhodophyta), or green (Chlorophyta). Their chemical and nutritional compositions are influenced by several factors such as species, geographical origin, seasonal, environmental and physiological variations, and processing method, among others.

Macroalgae

Seaweeds are well recognized as a rich source of polysaccharides, dietary fibre, minerals, vitamins as well as proteins, lipids, polyphenols, and many other phytochemicals. Moreover, they contain a variety of bioactive compounds including secondary metabolites with antibacterial, antiviral, antifungal, and antioxidant properties. Due to their diverse biological activities, marine macroalgae have gained increasing attention as a promising source for functional food and pharmaceutical products. Most Asian countries incorporate seaweed into their diet as a food ingredient due to their functionality and beneficial health properties. According to United Nations Food and Agriculture Organization (FAO) statistics, the annual harvest of macroalgae from wild and cultivated crops was 28.4 million tons in 2014, which accounts for nearly 40% of the global production.

Microalgae are microscopic organisms (<20 μm) and are often identified as phytoplanktons, including blue-green algae (phylum Cyanobacteria, class Cyanophyceae), diatoms (phylum Ochrophyta, class Bacillariophyceae), dinoflagellates (phylum Myzozoa, class Dinophyceae), as well as green and yellow-brown flagellates like chlorophyta, prasinophyta, prymnesiophyta, cryptophyta, and others. Among these, edible blue-green algae – *Nostoc*, *Arthrospira (Spirulina),* and *Aphanizomenon* species – have been used as food for many centuries. Being the basis of the marine food chain, microalgae play a key role in the productivity of marine environments and also produce numerous bioactive compounds. They have a rich nutritional profile of polyunsaturated fatty acids (PUFA) and carotenoids and chlorophyll pigments. Applications of the bioactive compounds of microalgae in both food and pharmaceutical industries have been expanded over the past decades. For example, *Spirulina* (*Athrospira platensis*) are widely used as a health food while *Dunaliella salina*, which have high concentrations of vitamin A and lipids, are cultured in commercial scale.

Polysaccharides from Macroalgae

Seaweeds are rich sources of polysaccharides (especially as sulfated polysaccharides) and display physicochemical and biological features that have potential functions for different applications. Large amounts of polysaccharides in seaweeds can be found in cell wall structure and as storage polysaccharides. The cell wall polysaccharides mainly consist of cellulose and hemicelluloses, neutral polysaccharides, and provide rigidity to the thallus in water.

The carbohydrate type varies greatly among algal species. Typical polysaccharides in green algae contain sulfated polysaccharides, sulfated galactans, and xylans; while brown algae varieties consist of fucoidan (sulfated fucose), laminaran (β-1,3 glucan), cellulose, alginates, sargassan, and mannitol. Meanwhile, red algal polysaccharides consist of agars, carrageenans, xylans, floridean starch (amylopectin-like glucan), water-soluble sulfated galactan, as well as porphyran as mucopolysaccharides (Figure 1). Polysaccharides with sulfated hemiester groups attached to sugar units are found in the form of "fucoidan" in brown algae (Phaeophyceae), as galactans (agars, carrageenans) in red algae (Rhodophyceae), and as arabinogalactans with lesser amount of sugars

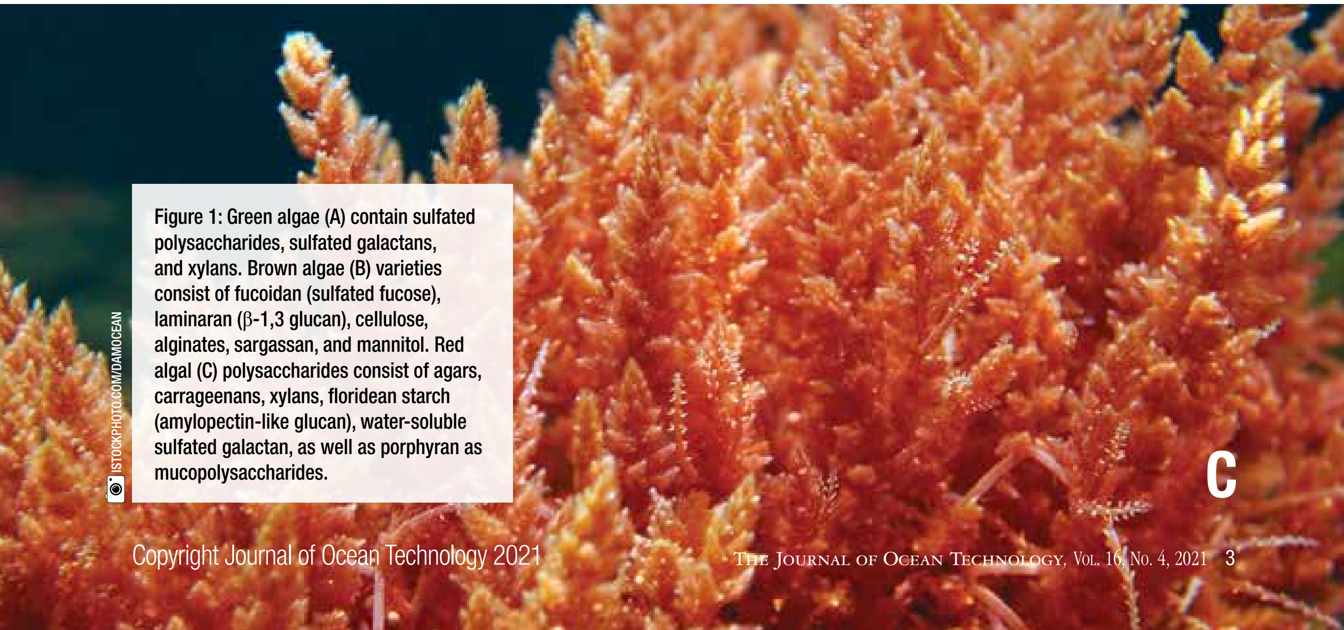

Figure 1: Green algae (A) contain sulfated polysaccharides, sulfated galactans, and xylans. Brown algae (B) varieties consist of fucoidan (sulfated fucose), laminaran (β-1,3 glucan), cellulose, alginates, sargassan, and mannitol. Red algal (C) polysaccharides consist of agars, carrageenans, xylans, floridean starch (amylopectin-like glucan), water-soluble sulfated galactan, as well as porphyran as mucopolysaccharides.

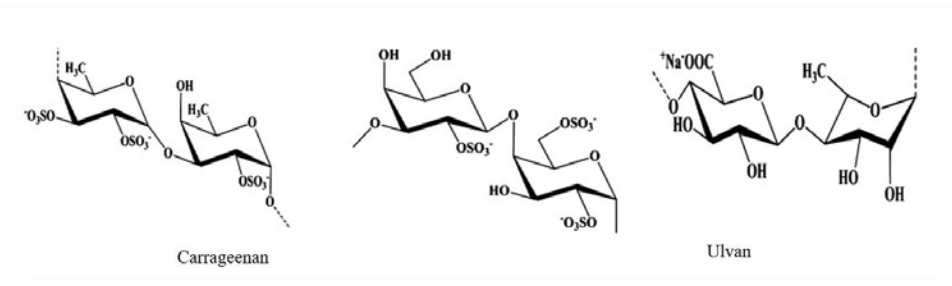

Figure 2: Monomeric units of antioxidative sulfated polysaccharides from marine algae.

in green algae (Chlorophyceae). Considering fucoidans, they further possess three subclasses, namely fucoidans, ascophyllans, and sargassan. Moreover, commonly occurring sulfated polysaccharides can be categorized into three different groups as water-soluble fucan or fucoidan obtained from brown algae (fucose, xylose, uronic acid, galactose); linear galactans and carrageenans obtained from red algae and water-soluble; and ulvan obtained from green algae (consists of sulfated rhamnose and sulfated aldobiouronic acid). Most of these classifications are based on the marine algal source and type of sugars present.

The total polysaccharide concentrations in seaweed species range from 4 to 76% on a dry weight basis while green seaweed species such as *Ulva* always account for a high content of polysaccharides. Most of these polysaccharides are considered as dietary fibres as they are not digestible by the human gastrointestinal tract and total fibre content of seaweeds ranges from 29.3 to 62.3 g/100 g. These dietary fibres promote intestinal health by creating an excellent intestinal environment. Edible seaweeds contain 33-62% total fibres on a dry weight basis and, compared to terrestrial plants, these fibres are rich in soluble fractions. Furthermore, these dietary fibres can be classified into two types: insoluble such as cellulose, mannans, and xylan; and water-soluble dietary fibres such as agars, alginic acid, furonan, laminaran, and porphyran. When considering the nature of soluble seaweed fibres, their passage through the gastrointestinal tract occurs largely without digestion and, due to their bulking capacity, they can improve the feeling of satiety. For example, alginates from brown seaweeds have been involved in water binding, fecal bulking, and decreasing transit time in the gut and this helps to prevent colon cancer.

Moreover, these algal fibres promote control and maintenance of intestinal flora and thereby dietary fibres show their potential as possible prebiotics. Sulfated polysaccharides can also act as prebiotics, hence exerting growth-promotion of beneficial bacteria in the gastrointestinal tract. Sulfated polysaccharides (SPs) isolated from marine algae comprise a complex group of macromolecules with a wide range of important biological activities that vary according to the species of algae. For example, fucoidan in brown algae (Phaeophyceae), carrageenan in red algae (Rhodophyceae), and ulvan in green algae (Chlorophyceae) (Figure 2) are some of the common SPs that are widely known as potential products with antioxidant activity. Moreover, direct by-products of alginates from edible brown seaweeds are considered to be sulfated polysaccharides.

As mentioned earlier, these sulfated polysaccharides obtained from marine algae can be classified according to the marine algal source or type of sugars present and also on the basis of the functional groups attached to the central sugar backbone. Most abundant SPs can be classified into three major groups by considering all of the above determination factors.

a) Water-soluble fucan or fucoidan – from brown algae – consist of fucose, xylose, uronic acid, galactose with varying degrees of sulfation.

b) Linear galactans and carrageenans – from red algae – consist of sulfated galactose and 3,6-anhydro galactose.

c) Water-soluble, ulvan – from green algae – consist of sulfated rhamnose and sulfated aldobiouronic acid.

Apart from antioxidant activity, these SPs possess anticoagulant, antiproliferative, antitumoral, anticomplementary, anti-inflammatory, antiviral, and antiadhesive activities due to their unique physicochemical characteristics. Most of these diverse biological activities are determined by glycan conformation of polysaccharides. Furthermore, several research studies have revealed that factors including molecular weight, sulfate content and distribution (sulfation patterns), introduction of other functional groups, and monosaccharide composition together with their unique structures are responsible for these wide array of biological activities.

Apart from fucoidan series of complexes, the other structural and storage polysaccharides, including agar, carrageenans, and alginates, are the most commercially exploited components in seaweeds. They possess textural and stabilizing properties that are widely utilized as thickening agents, forming gels, forming water-soluble films, and stabilizing products in the food industry. Polysaccharides from macroalgae have yet to be exploited in food and pharmaceutical applications; however, they exhibit numerous health benefits associated with important biological activities.

Proteins, Peptides, and Amino Acids from Macroalgae

Macroalgae are considered as one of the major sources of marine-derived proteins and peptides with biological properties. These exist as components of cell wall, together with enzymes, and bound with pigments and polysaccharides. The protein fraction of macroalgae differs depending on the species, season, geographic distribution, and cultivation conditions. Green and red seaweeds contain higher amounts of protein (10-47% of dry weight) compared to brown seaweeds (3-15% of dry weight). Even though there are clear variations in concentrations of essential amino acids, numerous studies have revealed that they contain all essential amino acids. The levels of amino acids present in seaweeds are comparable to those of the FAO and World Health Organization requirements of dietary proteins. Thus algae can also be used as an alternative protein source.

Furthermore, seaweeds contain an abundance of acidic amino acids, aspartic, and glutamic acids while lysine, tryptophan, cysteine, and methionine are considered as limiting amino acids. For example, leucine, valine, and methionine are abundant in *Palmaria palmate* (dulse) and these essential amino acids are closer to the protein composition in ovalbumin. Moreover, most seaweeds contain high levels of protein, similar to terrestrial plants such as legumes.

Structural, compositional, and sequential properties of bioactive peptides from marine algae have gained much attention due to their wide usage in food and pharmaceutical industries. The extraction of these bioactive peptides from most seaweed is difficult mainly due to the presence of large amounts of polysaccharides in their cell walls. However, enzymatic hydrolysis is widely used in food and pharmaceutical industries compared to solvent extraction and microbial fermentation. For instance, in order to improve peptide extraction from seaweeds, digestive enzymes or proteolytic microorganisms have often been used for enzymatic hydrolysis. Novel peptides were identified during protein extraction from seaweeds by enzymatic hydrolysis. Bioactive peptides usually contain 2-20 amino acid residues, but this could be even 40 residues or so. Amino acid composition and sequence determine their biological activities including antihypertensive, hypocholesterolemic, and antioxidant properties.

Presence of hydrophobic amino acids such as leucine, valine, and proline is common in the majority of peptide sequences of marine-derived peptides that exhibit antioxidant properties. These peptide sequences determine the bioactivity of particular proteins. Antioxidant peptides, such as carnosine and glutathione (generally present in animal muscle), have also been isolated from marine microalgae. Furthermore, protein hydrolysates from macroalgae are one of the best potential antioxidants of marine origin. As the main components of free amino acid from seaweeds, alanine, aminobutyric acid, taurine, omithine, citruiline, and hydroxyproline have been found and their levels depend on the species of seaweed. Among those amino acids, taurine (2-aminoethanesulfonic acid) has been identified as a vital amino acid for several biological activities including conjugating bile acids, regulating blood pressure, and acting as antioxidant, anti-inflammatory, and hypocholesteromic agents. Moreover, numerous seaweed species also contain unusual amino acids such as mycosporine-like amino acids and they are well known for their antioxidant properties.

However, phycobiliproteins (phycoerythrin, phycocyanins, and allophycocyanins) and lectins are the two major classes of functionally active algal proteins that have mainly been studied. Phycobiliproteins are protein-pigment (phycobilin) complex and considered as greatly soluble fluorescent proteins which are widely used as fluorescent markers in biotechnological applications and as natural colourants for food and cosmetic industries. Phycobiliproteins are mainly present in the cell wall of red seaweeds and are involved in the photosynthesis process by transferring fluorescence resonance energy. Furthermore, some of these phycobiliproteins were found to possess antioxidant, anti-inflammatory, antiviral, neuroprotective, hypocholesterolemic, and lipase inhibition activities. Macroalgal lectins are generally bound with carbohydrates and glycoproteins and have also been isolated from red algae and green algae (*Ulva* sp.). Bioactive lectins are involved in many biological processes including intercellular communication, antibacterial, antiviral, and anti-inflammatory activities as well as recognizing and binding carbohydrates.

The digestibility of seaweed proteins is limited due to the presence of high phenolic contents, particularly in brown algae. In contrast, most of the green and red algae have high protein availability as they possess low amount of phenolics. Apart from phenolic compounds, protein digestibility can also be affected by the presence of high amounts of polysaccharides, dietary fibres, and lectins as well as the level of protein glycosylation. Thus, enhancement of digestibility of algal proteins can be achieved by removing polysaccharides using processes based on an enzymatic treatment. However, more studies are needed to discover the *in vivo* digestibility of seaweed proteins while innovating efficient methodologies for increasing the bioaccessibility algal proteins.

Lipids Derived from Macroalgae

The main classes of lipids in seaweeds are phospholipids and glycolipids which comprise 1-5% of cell walls. Though seaweeds contain relatively low levels of lipids compared to other terrestrial plants like soy and sunflower, the availability of PUFAs are high. The favourable ratio between omega-3 and omega-6 in macroalgae promotes it as a dietary supplement and, due to the presence of essential fatty acids, they can be included as part of a balanced diet. The lipid content and fatty acid composition of seaweeds vary depending on species, geographical location, season, temperature, salinity, and light intensity. Studies on fatty acid accumulation in seaweeds have revealed that at lower temperatures algae are able to accumulate PUFAs. Eicosapentaenoic acid (EPA) is the predominant fatty acid in a majority of seaweeds, accounting for more than 50% of the total fatty acids content. Red and brown algae are rich in EPA, α-linolenic acid, and linoleic acid while green seaweeds are abundant in hexadecatetraenoic (n-3), oleic, and palmitic acids.

Figure 3: Processed seaweed products contain n-3 and n-6 fatty acids, thereby considered as a good source of polyunsaturated fatty acids.

EPA and docosahexaenoic acid (DHA) are found at high levels in various species of macroalgae. However, contribution of seaweeds as a food energy source appears to be low due to their lower lipid content (4.5% on a dry weight basis). Nevertheless, even the processed seaweed products (Figure 3) contain (canned and dried) protein, ash, n-3 and n-6 fatty acids and, thereby, they are considered as a good source of EPA and an important source to supply n-3 PUFAs for the maintenance of health. Moreover, the amount of phospholipids in various red seaweed species varies from 10 to 21% of the total lipid content and they contain almost equal quantities of glycoplipids such as monoglycosyldiacylglycerol (MGDG), diglycosyldiacylglycerol (DGDG), and sulfaquinovosyldiacylglycerol. MGDG and DGDG are the major glycoplipids in green algae.

The growing interest of research groups and consumers towards PUFA-rich lipid products has led to the discovery of alternative extraction techniques for lipids from seaweed-like marine sources. Among them, extraction of PUFA with ethanol from seaweeds has resulted in higher yields of PUFAs.

Microalgae

Microalgae (Figure 4) are microscopic marine organisms (< 20 µm), which are considered to be the most primitive and simply organized members of the plant kingdom. They can be found in both benthic and littoral habitats and in the ocean as phytoplanktons. These phytoplanktons are often comprised of blue-green algae, diatoms, dinoflagellates, and green as well as yellow-brown flagellates. Among these phytoplanktons, *Cyanophyceae* (cyanobacteria, blue-green algae) are oxygenic photosynthetic prokaryotes that show a large diversity in their morphology, physiology, ecology, biochemistry, and other characteristics. *Bacillariophyceae* (diatoms), dinoflagellates, and green and yellow-brown flagellates also possess most of the photosynthetic pigments as those of land plants. Thus, microalgae play a

Figure 4: Microalgae are microscopic marine organisms (< 20 µm) which are considered to be most primitive and simply organized members of the plant kingdom. They can be found in both benthic and littoral habitats and in the ocean as phytoplanktons.

vital role in the marine food chain by being one of the major photosynthetic organisms in the marine environment.

Moreover, they are abundant in bioactive compounds and biochemicals, such as PUFAs, and pigments, such as carotenoids and chlorophylls, antioxidants, polysaccharides, sterols, and vitamins. However, almost all these bioactive compounds present in microalgae are not desirable compounds for human consumption as some of them may produce strong hepatotoxins or neurotoxins. Therefore, before they can be used in human consumption, their safety should be assured.

Other Bioactive Compounds from Marine Algae
Pigments, phenolic compounds, minerals, vitamins, and antioxidants are some of the other vital bioactive compounds of algal origin. Most of the algal pigments are photosynthetic compounds that are used by autotrophs to capture solar energy for photosynthesis. The major categories of photosynthetic pigments in marine organisms are carotenoids, phycobiliproteins, and chlorophylls. Carotenoids (Figure 5) are a group of fat-soluble pigments performing a role as a light energy harvester in photosynthetic marine organisms. Moreover, they are potent antioxidants that inactivate reactive oxygen species. Green algae include β-carotene, lutein, violaxanthin, neoxanthin, and zeaxanthin; while red algae contain mainly α- and β-carotenes, lutein, and zeaxanthin; while β-carotene, violaxanthin, and fucoxanthin are predominant in brown algae. Fucoxanthin is one of the most abundant of all carotenoids and exhibits antioxidant, antiangiogenic, antiviral, antidiabetic, antiphotoaging, and anticancer properties while also possessing anti-obesity properties.

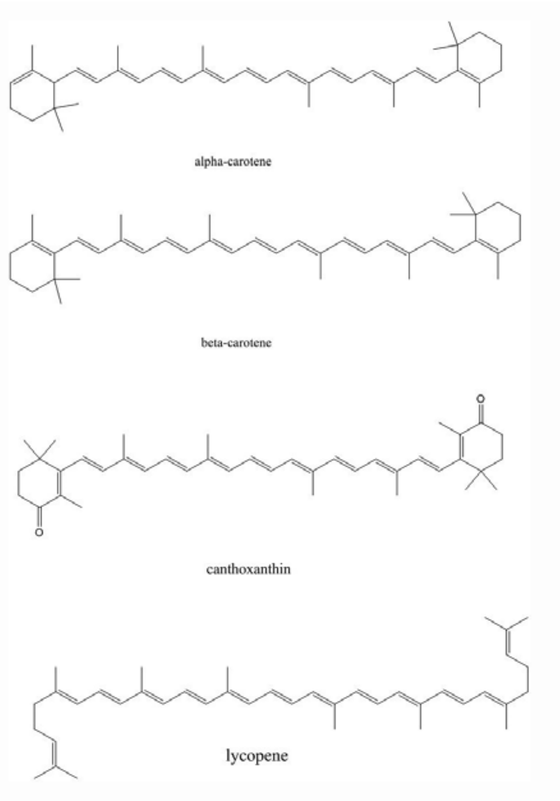

Figure 5: Chemical structures of common carotenoids found in marine algae.

β-carotene (with provitamin A activity) possesses the ability to prevent the onset of cancers as well as diminish the risk of cardiovascular and opthalmological diseases. Relatively high concentrations of β-carotene are present in microalgal species and have been widely employed in the food sector. Their antioxidant properties retard the harmful effects of free radicals and microalgal-derived β-carotene is found to be more biologically active than synthetic β-carotene. Tocopherol is another strong antioxidant which is found in all species of seaweeds and has the potential to reduce or prevent cardiovascular diseases.

Chlorophylls are lipid-soluble green pigments that are mainly used as natural colourants in food and beverages. Chlorophylls may undergo conversion in processed vegetable food and following ingestion by humans. In addition, chlorophylls and their derivatives possess other biological activities such as anticancer properties.

As pigments, marine algae are also a good source of vitamins. For instance, vitamin B (B1, B2, and B12) is present in red algae, while vitamins C and E are included in green algae. All macroalgae contain vitamin C (ascorbic acid) and red algae is widely used to prevent scurvy caused by vitamin C deficiency. More than the daily requirement of vitamins A, B2, B12, and two-thirds of the vitamin C requirement are provided by 100 grams of seaweed. Microalgae are considered as a good source of vitamins such as vitamins A, B1, B2, B6, B12, C, and E.

Phenolic compounds are abundant in marine algae and this makes marine algae one of the highly potent bioactive resources in the marine environment. Phenols consist of a hydroxyl group (–OH) bonded directly to an aromatic hydrocarbon moiety, and algal polyphenols are derived from polymerized phloroglucinol units (1,3,5-trihydroxybenzene). Seaweed species such as *Undaria* sp., *Laminaria* sp., and

Fucus sp. (Phaeophyceae, brown algae), and *Porphyra* sp. (Rhodophyta, red algae) are rich sources of polyphenolic compounds. However, brown algae also have high concentrations of phenols compared to green and red seaweed. This is mainly due to the presence of a high amount of phlorotannin in brown algae. Phlorotannin is responsible for several biological activities including antioxidant, anti-inflammatory, antidiabetic, antitumor, antihypertensive, and antiallergic activities. The extractable polyphenol levels from algae are lower than that of other phytochemicals. In addition, phlorotannins may serve as potential and unique natural antioxidants for use in functional foods, cosmetics, and pharmaceuticals at an industrial scale. Moreover, phenolic compounds present in marine algae have been identified as successful alternatives to synthetic antioxidants that can be employed to hinder the lipid oxidation in fish and fish by-products.

Apart from pigments, phenolic compounds, vitamins, and antioxidants, marine algae are also known to be high in essential minerals (Ca, Mg, Na, P, and K) and trace minerals (Zn, I, and Mn). Presence of these minerals is related to their ability to maintain their inorganic substances from sea water. Mineral compositions of marine algae depend on species and these may vary according to seasonal, environmental, geographical, and physiological factors. Besides, it is reported that marine algae contain 10-100 times higher mineral content than terrestrial plants. Hence, some marine algae are widely used as food supplements to fulfill the requirement of dietary intake of some minerals and trace elements. Moreover, due to the balanced content of Na and K, particularly in green algae (*Ulva rigida*), they may have a positive influence on preventing hypertension. In addition, numerous studies have revealed that seaweeds are excellent sources of calcium and have high iodine content. Thus, intake of marine algae or marine algae incorporated products provides a plethora of health benefits including prevention of several diseases such as hypocalcia symptom and osteoporosis cretinism, goiter, and mental defects. Therefore, necessary efforts should be taken to expand on their nutritional formulations in novel foods as well as to evaluate the claimed health benefits associated with those new products.

Dr. Tharindu R.L. Senadheera obtained her PhD in food science from Memorial University of Newfoundland in June 2021. Her PhD research was mainly focused on identification and characterization of marine derived bioactive molecules and their nutraceutical potentials. As a PhD scholar, she has worked alongside the Bioprocessing Research Facility at the Centre for Aquaculture and Seafood Development, Marine Institute, for various marine bioprocessing projects. Her research interests include characterization of marine derived phytochemicals, marine food product development, and value added utilization of marine by-products.

Dr. Deepika Dave is a research scientist in the Centre for Aquaculture and Seafood Development (CASD) at the Marine Institute of Memorial University of Newfoundland; and is a cross-appointed professor (biochemistry) at Memorial. Dr. Dave has over 22 years of increasingly responsible experience in engineering, science, management, research, training, and facilitation. She specializes in the areas of marine bioprocessing and bioconversion; blue biotechnology; and waste utilization (including production of high value molecules such as nutraceuticals, cosmetics, and pharmaceuticals from marine unutilized resources, both on small and pilot scales); environmental engineering (including waste characterization, water/wastewater treatment, management and utilization, remediation of contaminated air, water and soil); and energy production and management (including characterization of biomass, size reduction, production of bioethanol and biodiesel). She is a principal investigator for the delivery of product and process design, industrial, and development services at MI's Marine Bioprocessing Facility. Dr. Dave is involved in supervising/co-supervising graduate students enrolled within the Biochemistry and Fisheries Science program.