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# Sulfated and Non-Sulfated Polysaccharides from Seaweeds and their Uses: An Overview

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## Abstract

Polysaccharides are the major components in seaweeds. They are made up of several monosaccharides joined by glycosidic bonds, with or without sulfate and other groups in their structures. They can be broadly grouped into sulfated and non-sulfated. Their structural features offer interesting functional properties and hence serve as valuable additives in food processing, pharmaceuticals, cosmeceuticals, and other applications. This article gives a concise information on major sulfated and non-sulfated polysaccharides available from seaweeds and their uses.

***Keywords:*** *Sulfated Polysaccharides; Non-Sulfated Polysaccharides; Seaweeds*

## Introduction

Marine macroalgae, popularly known as seaweeds, are classified into three main groups based on the nature of their photosynthetic pigments, namely: red, brown and green, which are referred as Rhodophyceae, Phaeophyceae and Chlorophyceae, respectively [1,2]. Seaweeds are rich in fiber, several minerals, including iodine, polyunsaturated fatty acids (PUFA), proteins, essential amino acids, polysaccharides, and other nutrients. They also contain various types of metabolites such as sesquiterpenes, phlorotannins, bromoditerpenes, halogenated furanones, lectins, and other compounds, which are released by the algae to protect them against various adverse conditions they face. Seaweeds, therefore, are promising source of diverse biological activities [3].

Polysaccharides are the major components of seaweeds, contributing as high as 70% of their dry weights. The red algae (Rhodophyceae) contain the polysaccharides, agar, carrageenans, xylans, floridean starch (amylopectin-like glucan), water-soluble sulphated galactan, as well as porphyran. Brown algae (Phaeophyceae) contain alginic acid, and laminarin and sulphated heteropolysaccharide sargassum, while green algae (Chlorophyceae) have sulphated ulvans and xylans, a group of hemicelluloses [1,4]. The biological functions of polysaccharides relate to providing structure to the plants, physically supporting the thallus in water. Besides, their functions involve cell-cell recognition, stimulating host defense and hydration of intracellular fluid. Beneficial applications of polysaccharides and other seaweed compounds in diverse fields such as food processing, water treatment, fertilizer, bioremediation, bio-pesticides, and others have been discussed in many articles [5-11].

### Basic chemistry of polysaccharides

Chemically, polysaccharides are made up of several monosaccharides joined together by glycosidic bonds. They may be heteropolysaccharides (glycans) if the monosaccharides are of different types, or homopolysaccharides, if made up of only one type of

sugar unit. If the glucose is the only sugar unit, they are called glucans. Polysaccharides differ in the type of constituent unit, nature of glycosidic linkages, degree and type of branching, length of chains, molecular mass, and conformation. The most common constituent is D-glucose, but D-fructose, D-galactose, D-galactose, D-mannose, L-arabinose, and D-xylose are also frequent. The common functional group of polysaccharides is the hydroxyl group, which may sometimes be methylated or converted to sulfate esters or ketals, formed with pyruvic acid. Other derivatives found in polysaccharides include the amino sugars (D-glucosamine and D-galactosamine) as well as their derivatives (e.g. *N*-acetylmuramic acid), and sugar acids (glucuronic and uronic acids). Glycosides, particularly of phenolic compounds, are widely distributed in plant tissues. Derived compounds from polysaccharides can be several such as carboxy methyl cellulose, cellulose nitrates, hydroxy alkyl cellulose, methyl cellulose (all from cellulose); acetate, adipates, phosphates, succinates, carboxy methyl, hydroxyl ethyl, hydroxyl propyl, cationic salts (from starches), carboxymethyl, hydroxyl propyl and several other compounds. Hydrolysis of a glycoside in an acidic solution releases the monosaccharide and the alcohol. Polysaccharides and oligosaccharides have reducing and non-reducing ends. The reducing end of an oligo- or polysaccharide is the one end not involved in a glycosidic linkage. The algal polysaccharides can be broadly grouped into sulfated and non-sulfated. Sulfated polysaccharides are fucoidans, agar and carrageenans and ulvans, while the most commercially important non-sulfated polysaccharide is alginate. Variations in their structural features offer diverse types of polysaccharides [12,13]. Rapid methods are available for their assays [14]. The contents of polysaccharides in some important seaweed species are given in table 1.

| Seaweed group | Genus | Polysaccharide contents (%, dry weight basis) |
|---|---|---|
| Brown algae | *Laminaria* | 38 - 68 |
| | *Fucus* | 62 - 66 |
| | *Ascophyllum* | 42 - 70 |
| | *Undaria* | 35 - 46 |
| | *Sargassum* | 49 - 62 |
| Green algae | *Ulva* | 15 - 65 |
| Red algae | *Chondrus* | 55 - 66 |
| | *Porphyra* | 40 - 54 |
| | *Gracilaria* | 36 - 63 |
| | *Palmaria* | 38 - 74 |

***Table 1:*** *Polysaccharide contents of some major seaweeds.*
*Source: Adapted from Reference [9].*

The structural features of polysaccharides including those brought about by chemical modifications strongly contribute to specific functional properties [15-17]. Polysaccharides are able to bind water up to 20 times their weight to give hydrogel, a property, which qualifies them to be referred as hydrocolloids or phycocolloids [18]. Many polysaccharides can form hydrogels by either heating or cooling. The gel is composed of at least two components, where a polymer forms a three-dimensional network in a liquid medium such as water. Formation of gel involves non-covalent interactions, such as hydrogen bonding, hydrophobic and ionic interactions among the constituents and are formed from cooling of heated solutions of polymers at low concentrations ranging from 0.1 to 15% (w/w). Gelation of polysaccharides is reversible; melting of gel is therefore possible by heating and can influence water holding capacity and flavor release by the gels [19,20]. Structural features influence rheological properties of polysaccharides The gels can be rigid, flowing, brittle, sparingly, firm, soft, spreadable, sliceable, rubbery, or granny, depending upon the degree of interactions of polymers and ration [21]. They are also able to interact macromolecules (proteins, polysaccharides and lipids) and also with small molecules such as ions, colorants, flavors, fatty acids, vitamins, biosurfactants, and phytochemicals. These interactions also favor formation of emulsions and foams [22]. The above

functional properties make polysaccharides valuable food additives as thickening and texture modifiers, stabilizers, water retention compounds, emulsifiers, foam stabilizers, binders of ingredients, and modifiers of viscosity. Polysaccharides are able to prevent syneresis and dryness, control starch retrogradation, enhance flavor, retard crystal growth, replace fat and improve satiety and fiber contents of foods These properties also impart them interesting biological activities [15-17]. This article is intended to briefly discuss major sulfated and non-sulfated polysaccharides of seaweeds, their characteristics and applications in food and pharmaceutical fields.

**Sulfated polysaccharides**

Marine algae are rich in polysaccharides that contain sulfate moieties in their structures. Such polysaccharides include carrageenans in red algae, fucoidans in brown algae and ulvans in green algae. Sulfated polysaccharides and their lower molecular weight oligosaccharide derivatives from seaweeds have been shown to possess a variety of biological activities such as anticoagulant, antiviral, anticancer, antioxidant, antitumor, immunomodulating, antihyperlipidemic and antihepatotoxic activities [23-27]. Furthermore, their structural features make them ideal materials for biomedical applications, such as cellular scaffold, coatings for biomedical disposals, treatments for different diseases, controlled release of drugs [28].

**Carrageenans**

Carrageenan is a generic term for a family of anionic polysaccharides, isolated from red seaweeds. In terms of their chemical structures, carrageenans are classified into kappa (k), iota (i) and lambda (λ) carrageenans. Other types such as μ-, α-, β-carrageenans, a hybrid form consisting of k- and i-carrageenans and other minor types are also available. The polysaccharide is a galactan consisting of galactose units. The backbone structure of carrageenan is based on linear chains of repeating galactose units in D configuration and 3, 6-anhydro-galactose copolymer by alternating α-(1-4) and β-(1-3) linkages (i.e., alternating (1-4)- α-D-galactopyranose and (1-3)- β-D-galactopyranose). Carrageenans contain 15 to 40% ester sulfate. The differences among the various forms of carrageenan are related to presence of 3, 6-anhydro-D-galactose (3, 6-AG) in the chain and the presence and position of sulfate groups. The κ-, ι- and λ- carrageenans are distinguished by the presence of one, two and three ester-sulfate groups per repeating disaccharide unit, respectively The κ -carrageenan is composed of D-galactose, 3, 6-AG and ester bound sulfate in a molar ratio of 6:5:7. The λ- carrageenan has no 3, 6-AG, but has three sulfate groups, whereas the ί -carrageenan is intermediate with a 3, 6-AG and two sulfate ester groups. Whereas in ί-carrageenan the hydro-galactose residue carries a sulfate group, it is absent in the κ-form. The β-carrageenan is devoid of sulfate content The relative presence of different carrageenans depends on the algal source, season of its harvest as well as on the extraction procedure used [25,29-31]. Figure 1 gives the chemical structures of κ-, ι- and λ- carrageenans.

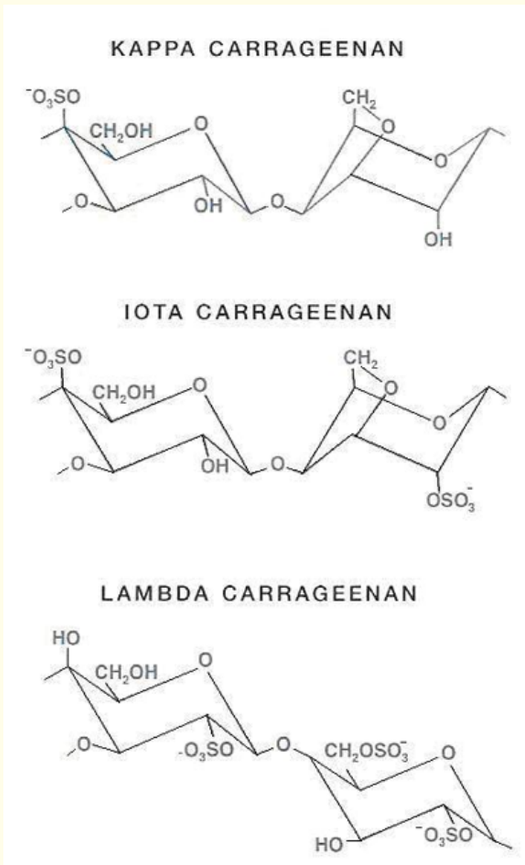


***Figure 1:*** *Structures of major carrageenans.*

Commercially carrageenans are extracted from *Eucheuma cottonii* and *E. spinosum, C. crispus* (known as Irish moss), *Gigartina stellata* and *Chondrus* spp [2]. The washed and dried seaweed is treated with dilute calcium or sodium hydroxide. It may be noted that concentration of alkali used for extraction and duration of treatment can affect yield, its sulfate content, viscosity and gelation properties of the polysaccharide. After alkali extraction the slurry is filtered employing a filter aid. The filtered liquor is centrifuged and concentrated by evaporation. Carrageenan can also be precipitated from the aqueous extract by isopropyl alcohol. Separated carrageenan is dried by freeze-drying or dried under vacuum. The extraction process has been suitably modified according to the species used and the type of carrageenan [31,32].

Carrageenans have molecular weights in the range of $10^5$ to $10^6$ kDa. Their solubilities in water depend upon their chemical composition, particularly sulfate content, presence of associated cations, and hydrophilic character. Being highly sulfated with no 3, 6-AG content, λ-carrageenan is easily soluble, whereas κ-carrageenan, with one sulfate group, is relatively less hydrophilic and, thus, less soluble in water. Aqueous solutions of ι-carrageenan tolerate high concentrations of electrolytes such as NaCl up to 20 - 25%, while k-carrageenan will be salted out. The κ and λ-carrageenans are soluble in hot (70°C) sucrose solutions up to 65%, while ι-carrageenan is not easily soluble in sucrose solution at any temperature. Acid and oxidizing agents may cleave glycosidic bonds in carrageenans leading to loss of properties [29]. Carrageenans can be characterized by rheological properties. While k- and i-carrageenans form gel, λ-carrageenan does not gel. Gelling temperatures ranged from 32 to 36°C for the κ-carrageenan and 70 - 74°C for ι-carrageenan. Monovalent cations, such as potassium, rubidium and caesium, strongly promote gelation of k-carrageenan. Difference in gelling and melting temperatures (known as 'hysteresis) is dependent on the type of carrageenans: For κ-carrageenan it is 15° to 20°C, and about 5°C for ι-carrageenan's. K-carrageenans from different seaweeds had gel strengths (G') of 4000 - 6500 Pa at 25°C. Viscosity of carrageenan solution is influenced by concentration, temperature, the presence of salts and other solutes and molecular weights. Commercial carrageenans are generally available in viscosities ranging from 5 to 800 cps Viscosities of k- and i-carrageenans at a concentration of 1.5% (w/v) is measured usually by rotational viscometers such as Brookfield at high temperature, such as 75°C to avoid the effects of gelation, while for the cold water soluble, non-gelling λ -carrageenan, viscosity is measured at 25°C. Salts reduce viscosity in the order $K^+ > Ca^{2+} > Na^+$. Hot k-carrageenan in solutions are able to interact upon cooling with other gums such as locust bean gum and konjac mannan, which is useful to modify food texture [32,33]. Some properties of carrageenans useful in food product development are compared in table 2.

| Properties | Kappa-carrageenan | Iota-carrageenan | Lamba -carrageenan |
|---|---|---|---|
| Molecular weight (kDa) | 100 - 1000 | 100 - 1000 | 100 - 1000 |
| Solubility in hot water | Soluble at > 60°C | Soluble at > 60°C | Soluble |
| Solubility in cold water | Sodium salt soluble<br>K and Ca salts insoluble | Sodium salt soluble<br>K and Ca salts give thixotrophic dispersion | Sodium salt soluble |
| Solubility in hot (80°C) milk | Soluble | Soluble | Soluble |
| Solubility in cold (20°C) milk | Na, Ca, Ki salts insoluble, but swells | Insoluble | Soluble, thickens |
| Gelation | Gels, strongest with $K^+$ | Gels strongest with $Ca^{++}$ | No gelation |
| Solubility in concentrated sugar solution | Soluble, when hot | Soluble with difficulty | Soluble, when hot |
| Solubility in concentrated salt solution | Insoluble | Soluble, when hot | Soluble, when hot |
| **Stability**<br>Freeze - thaw<br>pH > 5<br>Syneresis<br>Salt tolerance | <br>No<br>Stable<br>Yes<br>Poor | <br>Yes<br>Stable<br>No<br>Good | <br>Yes<br>Stable<br>No<br>Good |

***Table 2:*** *Comparison of properties of major kappa, iota and lambda carrageenans.*

*Source: Adapted from References [2,30,47].*

Carrageenan possesses promising bioactivities both *in vitro* and *in vivo*, showing promising potential to be developed as therapeutic agents Sulfated carrageenans have -inflammatory activity, and therefore able to activate macrophages and monocytes for treatment of diseases such as tuberculosis. The bioactivities of carrageenans have also been demonstrated against respiratory diseases, antitumor and immunomodulatory, anticoagulant, antiviral, and antioxidant activities. Hydrogel nanoparticles have gained considerable attention in recent years as one of the most promising nanoparticulate drug delivery systems owing to their unique potentials via combining the characteristics of a hydrogel system (e.g. hydrophilicity and extremely high water content) with a nanoparticle (e.g. very small size) [34,35]. Carrageenan-based pellets, beads, nanoparticles, microparticles, hydrogels, films, matrices and other devices can have roles as carriers for targeted drug delivery and tissue engineering [29,34-36]. Carrageenans are used in food products such as ice cream, chocolate milk, yogurt and soy milk, as thickeners and stabilizers taking advantage of their functional properties such as emulsification, gelation, complexation with polyelectrolytes, and other properties. Process variables such as temperature, pH, ionic strength, cations, additives such as proteins, and other hydrocolloids influence the texture of carrageenans [4,12]. Carrageenans can be complexed with other polymers such as chitosan, gelatin etc. to enhance their film forming and also drug delivery properties [37]. The presence of appreciable levels of sulfated polysaccharides as dietary fibre and also protein and biologically important fatty acids made *G. edulis* nutritionally important for human and animals [38].

**Agar**

Red seaweeds (Rhodophyceae) consisting of more than 700 genera and 6000 species are sources of agar, also referred as the Japanese gelatin, Japanese isinglass, vegetable gelatin and agar-agar. Of these, *Gracilaria* spp. and *Gelidium* spp. are conventional rich sources of agar; besides *Hypnea* spp [39-42]. In the Japanese extraction process a mixture of up to six or seven types of red seaweed (usually *Gracilaria,* and *Gelidium* spp) is boiled in acidified (pH 5 to 6) water containing bleaching agent for about 10 hrs. The slurry is filtered under pressure, cooled and the agar gel is cut into suitable sizes, which is further purified by repeated freezing and thawing. Agar from the extract may also be precipitated by alcohol. Agar contains 2 to 5% sulfate residues. The extraction method influences gel strength of agar due to structural changes such as degree of methoxylation and sulfation; alkali adversely affects sulfate and 3, 6-anhydrogalactose contents [42,43]. A novel photo bleaching method gave agar from *Gracilaria* spp having excellent gel strength [44].

Agar is composed of two polysaccharides, agarose and agaropectin. The basic monomer of agarose is galactose, and consists of alternating 1, 4-linked 3, 6 anhydro -α-L-galactopyranose and 1, 3-linked β-D galacto-pyranose. Agarose has a double helical structure; the double helix aggregates to form a three dimensional framework, which holds the water molecules within the framework during gelation [2]. Agaropectin is more complex in structure, and contains residues of sulfonic, pyruvic and uronic acids, in addition to D-galactose and 3, 6-anhydro-L-galactose, which differentiates between the agarose and agaropectin. These residues may influence the gelling property of agar [42,45]. The methods of structural analysis of agar include determination of monosaccharide constituents, partial depolymerization by reductive hydrolysis, identification of disaccharide repeating units by NMR spectroscopy, and sequence analysis by enzymatic degradation [46]. FTIR-ATR and FT-Raman spectroscopy techniques are able to identify seaweeds as sources of agar and other polysaccharides. [47]. It is important to note that during alkali extraction the contents of 3, 6-anhydro galactose are not affected to get maximum gel strength. Longer alkali treatment has adverse effects on the polysaccharide yield and gel strength [48]. Figure 2 shows the structure of agar.

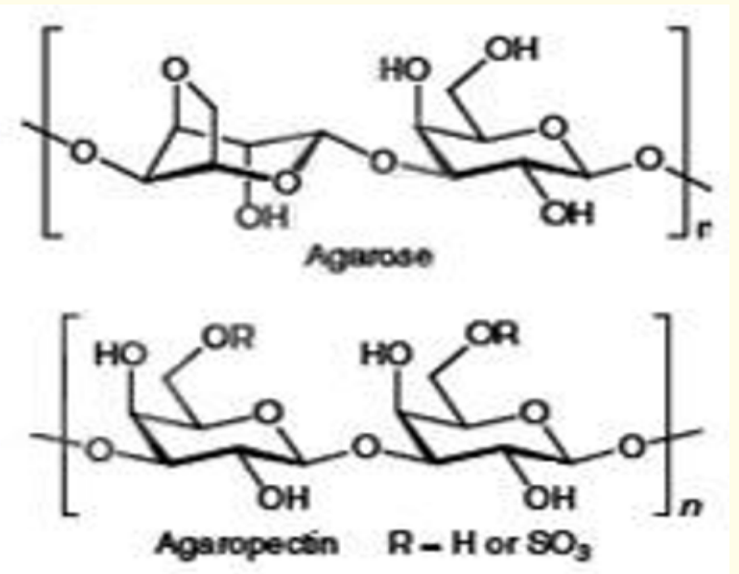


***Figure 2:*** *Structure of agar.*

Commercial agar is white, shiny, semi-transparent, tasteless, and odorless having less than 20% moisture. It is insoluble in cold water, soluble in boiling water. Hot (75° - 80°C) 1.5% aqueous agar is clear and congeals at 32° to 39°C to a firm resilient gel, which becomes liquid only above 80°C. Gelation of agar is due to coil-helix transition followed by aggregation of helices, holding water molecules within the interstices. The gel strength of agars from various *Gracilaria* spp. ranged between 260 to about 1920 g $cm^{-2}$. Agarose has higher gelling ability than agaropectin [2,40,41,44,45]. Interaction of agar with sugar increases the strength of the gel, by a phenomenon called sugar reactivity Difference in gelling and melting temperatures of agar, known as 'hysteresis', makes agar a popular thickener, lubricant, emulsifier and water absorbent in food, microbiological and pharmaceutical fields. Food grade agar at about 1 - 2% is used as a stabilizer and texturizer in food items such as canned meat, confectionery, and for glazing and icing in the baking industry. It can replace gluten in wheat products, and also gelatin and can control syneresis in frozen stored foods. The non-digestible nature of agar also makes it as fiber [4,48]. Agar is used as a laxative and for treatment of constipation. Oligosaccharides are attracting increasing interest as functional food ingredients such as prebiotics. They can be extracted or obtained by enzymatic hydrolysis of polysaccharides. Oligosaccharides from agar have been reported to possess antioxidant properties and ability to suppress the production of a pro-inflammatory cytokine. Agar is used in microbiology, gel electrophoresis, chromatography, immunology, and biotechnology. A hydrogel system with superior absorbency and pH resistance developed based on polyacrylamide-grafted blend of agar and sodium alginate has potential applications in health care and other fields [49].

## Fucoidans

Fucoidans (fucans) are a group of polysaccharides that are widely found in the cell walls of brown seaweeds including *Sargassum* spp. and *Fucus vesiculosus* [25,28]. *S. muticum* and *Saccorhiza polyschides* contain fucoidans and also alginates in appreciable quantities [50]. Fucoidan are extracted by ambient temperature or hot (60° - 70°C) water treatment of the seaweed. While the G-fucoidan is mostly extracted at room temperature, U-fucoidan is extracted at 70°C. The crude fucoidan is purified by hydrophobic chromatography, followed by precipitation with cetyl trimethyl ammonium hydroxide or cationic cetylpyridinium chloride. Fractionation with the cationic detergent, cetrimide, allows better separation of the two fucans. Fucoidans essentially consist of α--L-fucose units (usually referred as α-L-fucopyranose). They also contain sulfate with small proportions of uronic acids, galactose, xylose, arabinose and mannose, glucose, and xylose. They can have one of two types of homofucose backbone chains, with either repeated (1-3)-linked α-L-fucose residues or alternating (1-3) - and (1-4)-linked α-L-fucose residues U-fucoidan contains glucoronic acid, approximately 20% of its weight The G-fucoidan contains significant amounts of D-galactose, besides fucose, while uronic acids are present in uronofucoidan, both class containing low proportions of sulfate groups. They may be sulfated at $C_4$, $C_2$ or both $C_2$ and $C_4$ positions of fucose units [28]. The manufacture, characterization and biological transformation of fucoidans from macroalgae have been discussed [28,51-53]. The structure of fucoidan sis depicted in figure 3.

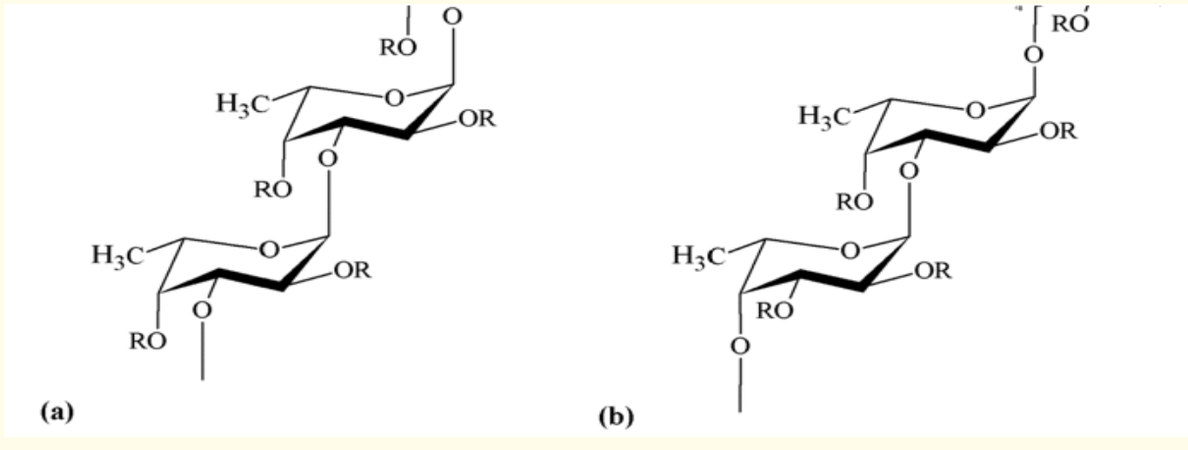


***Figure 3:*** *Structure of fucoidan (a) The chain is composed of repeating (1-3)-linked a-fucose residues only (b) The chain consists of alternating (1-3)- and (1-4)-linked a-L-fucose residues. R represents attachment of carbohydrate residues (glucuronic acid, mannose, galactose, xylose, a-L-fucose) and non-carbohydrate (sulfate and acetate) groups.*

The solubility of fucoidan is related to the level of branching, depending on the content of sulfate groups. It is viscous in very low concentrations and susceptible to breakdown by diluted acids and bases. A sol-gel transition is induced by addition of glycerol in aqueous solution containing high concentrations of fucoidan. Mixed gels of fucoidan with gelatin and bovine serum albumin have soft texture. The commercially available fucoidan from *Fucus vesiculosus* is a heterogenous mixture of more than 15 different fucans with varied properties [29,55,56]. Fucoidans show various physiological and biological features, such as anticoagulant, antioxidant, antiviral, antimicrobial, anti-tumor, anti-mutagenic, anti-complementary, immune-modulating, and anti-inflammatory activities. The anti-thrombin activity of fucoidans is comparable with that of heparin [57]. These properties make fucoidans interesting ingredients in pharmaceutical industry [29,55-58].

**Ulvan**

Ulvan is a sulfated polysaccharide present in *Ulva* spp. such as *U. conglobate*, *U. lactuca* and *U. rigida*, the cell walls of which contain ulvan up to 29% ulvan on dry weight basis. *U. pinnatifida* (wakame) is globally one of the most commonly produced species for ulvan. Like most other polysaccharides, ulvan is also extracted with hot water. Its yield can be improved by the presence of calcium chelating agents, acid or alkali in the water. Ulvan with improved purity is obtained by precipitation with ethanol, and then concentrated by freeze-drying. The yield and specific composition of ulvan, however, depends on environmental factors, the seaweed, the season of collection and the extraction method employed [59,60].

Ulvans are highly charged sulphated polyelectrolytes, composed mainly of rhamnose, xylose, iduronic and glucuronic acid as the main carbohydrate constituents [25]. Besides, mannose, galactose and arabinose have also been found in some ulvans. Two major kinds of ulvans have been identified, namely, water-soluble ulvan and insoluble cellulose-like material. The average molecular weight of ulvans ranges from 189 to 8,200 kDa [28]. Ulvan exhibits antioxidant activity, depending on its molecular weight. Ulvan from *U. conglobate* has significant anticoagulant activity. It has also antiviral and immunomodulating activities and potential to treat gastric ulcers and for targeted drug delivery. The polyelectrolyte properties of ulvan may favor its potential uses in the biomedical field [28,61].

**Furcellaran**

Furcellaran, also called 'Danish agar', is obtained from the red seaweed, *Furcellaria fastigiata*. The polysaccharide is isolated from the dry seaweed by alkali treatment followed by hot water extraction, followed by vacuum concentration. Furcelleran can also be precipitated with 1-15% potassium chloride. The separated gel threads are concentrated by freezing, the excess of water is removed by centrifugation or pressing. The product contains 8 - 15% salt. Furcellaran is composed of D-galactose (46 to 53%), 3, 6-anhydro-D-galactose (30 to 35%) and sulfated portions of both the sugars. The structure of furcellaran is similar to k-carrageenan; the essential difference is that k-carrageenan has lower (1 to 5%) sulfate groups while furcelleran contains 16 to 20% ester sulfate [29].

Aqueous preparations of furcellaran form thermo-reversible gels. In comparison with carrageenans, the concentration required for a gel of defined properties follows the order i-carrageenan > k carrageenan > furcelleran. Gelation can be induced either by mono or divalent cations such as $K^+$, $NH_4^+$ and $Cs^+$ and involves formation of double helix, similar to k-carrageenan. $Na^+$ prevents gel formation. The gels retain good stability against food grade acids. Addition of sugar changes the gel texture from a brittle to elastic. Furcelleran is used as an emulsifier and stabilizer in food products. Furcellaran has the advantage over pectin in marmalades as it allows stable gel at sugar concentrations even at 50 - 60%. The required concentration of furcelleran for gelation is 0.2 - 0.5%, depending on sugar content and required gel strength. Gels of furcelleran containing milk are used in puddings, cake fillings and icings [29].

**Porphyran**

Seaweed species belonging to *Porphyra s*pp. contain the sulfated polysaccharide, porphyrin, up to 48% of their dry weight. Chemically it resembles agar. It has a linear backbone of alternating 3-linked β-D-galactose and 4-linked α-L-galactose-6-sulfate or 3, 6-anhydro-α-L-galactose units. It has been suggested to possess antihyperlipidemic, antioxidant and apoptotic activities [24].

**Non-sulfated polysaccharides**

**Alginate**

The term 'algin' or 'alginate' is used as a generic name for salts of the alginic acid such as sodium, potassium, ammonium, calcium and propylene glycol alginates. Algin occurs in the intracellular space of brown seaweeds in the form of insoluble mixed salts of mainly calcium, with lesser amounts of magnesium, sodium and potassium. The important algal sources of alginate include *Macrocystis* spp., *Ascophyllum* spp. and *Laminaria* spp., *Ecklonia* spp. and *Sargassum* spp. [47,62]. The two widely practiced methods for production of alginate are those of Green and Le Gloahec-Herter processes [2,63]. In the Green's process, fresh alga is demineralized with 0.3% aqueous HCl, followed by pulverization and then treatment with an aqueous 8 - 2.0% sodium carbonate at pH 11. The supernatant is heated to 50°C, filtered under pressure and then mixed with 10 - 12% aqueous $CaCl_2$, when the insoluble calcium alginate is precipitated. The alginate is bleached with a 10% aqueous sodium hypochlorite, drained and mixed with 5% HCl. The precipitated alginic acid is washed with water to remove the calcium. The purified alginic acid is converted to desired salt by treatment with appropriate carbonate, oxide or hydroxide, dried, grounded and packed [2,63]. Alginates and also laminarins and fucoidans can be separated by a simple hydrophobic chromatographic method [64].

Alginic acid has a molecular formula of $(C_6H_6O_6)_n$, where the value of 'n' varies from 80 to 83; with a maximum molecular weight of 200 kDa. Alginic acid contains units of D-mannuronic acid (M) and, L-glucuronic acid (G). The polymeric linear structure of alginic acid consists of β-(1-4)-linked D-mannuronic acid (M) and α-(1-4)-linked L-guluronic acid (G) residues. The blocks are composed of consecutive G residues (GGGGGG…), consecutive M residues (MMMMMM..) and alternating M and G residues (GMGMGM…) Increase in molecular mass of alginate was found to be growth-associated The ratio of D- mannuronic to L-glucuronic acids in alginic acids vary with type of seaweed, its age, portions of the seaweed used and its location. Newly synthesized alginate contains entirely poly-M sequence, which are subsequently converted to guluronic acid by the enzyme mannuronic acid-epimerase. The G: M ratio is usually in the range of 1.45 to 1.85. Biochemical and biophysical properties of alginate are dependent on the molecular weights and G: M ratios. G blocks are believed to be important in influencing binding alginate with $Ca^{++}$ and $H^+$ ions and thereby its gelation [65-67].

Alginic acid is essentially insoluble in water. Monovalent ions such as sodium and ammonium interact with the carboxyl groups of alginic acid to form water-soluble colorless, transparent salts, having a wide range of viscosity, which increases as a function of concentration, molecular weight, and characteristic G:M ratio. Divalent alkali metal ions such as $Ba^{+2}$, $Ca^{+2}$, $Mg^{+2}$ and $Sr^{+2}$ induce gelatin of alginate, which occurs without any heating or cooling. Presence of G units in the structure determine binding capacity of alginate with $Ca^{+2}$. Junction zones are formed due to interchain cross-linking by metal $Ca^{+2}$ cross-links. The structure of the alginate gels has been described by the so-called 'egg-box model' (Figure 4), in which each divalent cation (e.g. $Ca^{2+}$) is bound to the carboxyl and hydroxyl groups of four guluronate monomers from two adjacent chains of the polymer. During gelation, formation of strongly linked dimer associations is followed by the formation of weak inter-dimer associations mainly governed by electrostatic interactions [68]. Age and habitats of the seaweed are important in determining composition and hence gelation properties. Additives such as dextran and glycerol dramatically change the viscosity of alginate solution [65].

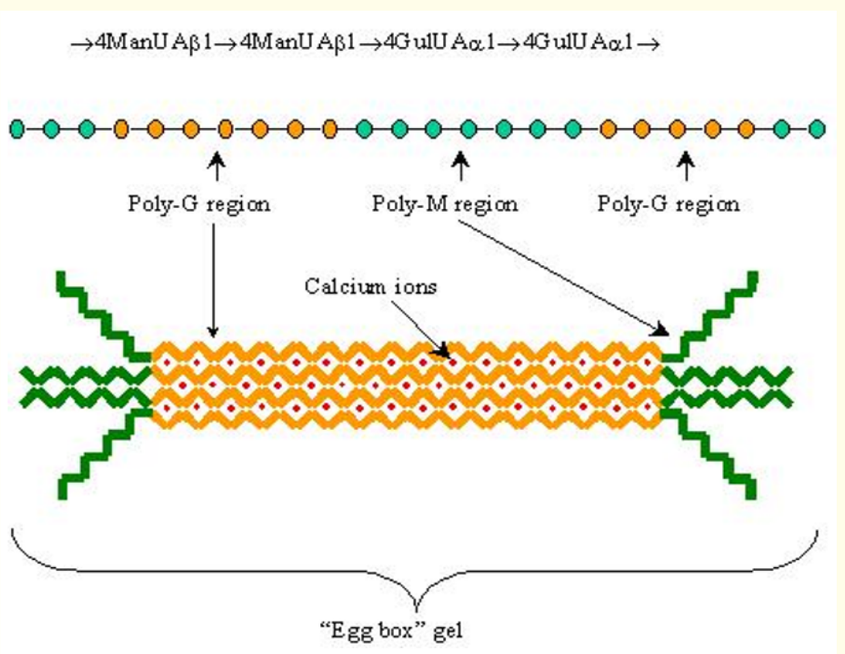


***Figure 4:*** *The 'egg-box' model of alginate gel formed in presence of calcium ions, Source: Rastall RA, Tailor-made food ingredients: enzymatic modulation of nutritional and functional properties, IFIS Publishing, https://www.ifis.org/, with permission.*

The bioactivities of alginate include antibacterial, pre-biotic, hypo-cholesterolemic, antihypertensive, anti-obesity, anti-ulcer activities. Alginates having high M contents are immunogenic and significantly more potent in inducing cytokine production compared with high G alginates. They also exhibit blood coagulation and platelet activation effects [9,16]. Alginic acid and alginates are used as thickening and stabilizing agents in a variety of food products Propylene glycol alginate, because of its solubility at low pH, is a popular thickener and emulsifier in low pH products such as sauces, syrups and sherbets [62]. Alginate forms film, which are popular because of its non-toxicity, biodegradability, biocompatibility, and low price [37]. Alginate gels can also be employed as matrix polymers for encapsulation of enzymes, drugs, proteins, cells and DNA and also for wound dressing. Alginate hydrogels are useful in wound healing, drug delivery, tissue engineering as these gels retain structural similarity to the extracellular matrices in tissues It is also useful as dietary fiber, Alginate is well recognized as scaffold useful to treat loss or failure of organs. These scaffolds can be reinforced with hydroxyapatite for better strength. Alginate gels promote blood vessel formation and also provide sustained and localized release of heparin binding growth factors [9,35,66,69].

**Laminarin**

Laminarin is found in the fronds of brown algae, Laminaria spp. such as *L. japonica, L. hyperborean* and to a lesser extent, in *Ascophyllum* spp., *Fucus* spp. and *Undaria* spp, which use it as a food reserve. These algae contain laminarin up to 30% on dry basis (2, 2012). Laminarin is a β-glucan, and has a molecular weight range from 2900 to 3300 Da containing between 50 and 69% of D-glucose and an average of 1.3% D-mannitol. Its linear structure is composed of β- (1-3)-D-glucose and some β-(1-6)-intra-chain links [16]. The process for laminarin extraction has been detailed [54,70].

Laminarin possesses anti-tumor, anti-apoptotic, anti-inflammatory, anticoagulant and antioxidant activities .The β-glucan also provides protection against infection by bacterial pathogens and adverse effects of exposure to ionizing radiations. Besides, it boosts the immune system, reduces serum cholesterol levels, and lowers systolic blood pressure [16,54]. The polysaccharide has been recognized as a regulator of intestinal metabolism through its impacts on mucus structure, intestinal pH, and short-chain fatty acid formation [9]. Laminarin is also a potential anticancer agent. It has also been shown to be a safe surgical dusting powder. Laminarin shows anticoagulant activity after structural modifications such as sulphation, reduction or oxidation. There is scope for modification of bioactivities of laminarin by suitable chemical modifications [9,70]; Laminarin does not gel or form viscous solution. Laminarin has applications in food product development, as fiber and also as a prebiotic [54,70,72]. Preparations containing β-D-glucans, laminarin and fucoidan are manufactured by the health industry and marketed for their beneficial properties on the immune system [16].

**Other polysaccharides**

Seaweed species also contain minor amounts of other polysaccharides such as funoran, ascophyllan and sargassum and also oligosaccharides and other sugar derivatives [16]. Oligosaccharides isolated from seaweed species and also from the degradation of polysaccharides such as agar exhibit immunostimulative, antioxidant, blood glucose as well as cholesterol lowering and antitumor activities, besides suppressing production of pro-inflammatory cytokine, activities. They have also functional role as prebiotics [7,16]. Isolation and physico-chemical characterization of a carbohydrate, known as floridean starch from agarophytic red algae have been reported. Unlike plant starches, floridean starch does not have amylase and has a low level of covalently linked phosphate [73]. Mannitol is a sugar alcohol which is present at 25% of dry weight of some brown algae, especially belonging to Laminaria, Ascophyllum and Ecklonia. The diverse applications of mannitol include pharmaceuticals, foods paint and varnish industry, leather and paper manufacture, plastics industry [16]. Table 3 summarises potential food applications of alginate, agar and carrageenans in diverse food products. Bioactivities and medicinal applications of major seaweed polysaccharides are summarized in table 4.

| Product categories | Polysaccharides | Potential applications |
|---|---|---|
| Baked goods | Alginate | Emulsifier |
| Beverages | | Fat replacer |
| Confectionery | | Water binding agents |
| Dairy | | Controls syneresis |
| Desserts | | Improves texture |
| Dressings and dips | | Creates creamy mouth feel |
| Fried foods | | Enhances fiber content |
| Frozen foods | | Antioxidant activity |
| Meat analogs | | Antimicrobial activity |
| Meat products | | Encapsulation of food flavors |
| Pasta | | Increase yield and hence reduces production costs |
| Restructured products | Carrageenans | Controls syneresis |
| Sauces and gravies | | Emulsifier |
| Snack foods | | Enhances mouth feel |
| Soups | | Improves viscosity |
| | | Antioxidant activity |
| | | Antimicrobial activity |
| | | Anti-browning activity |
| | | Moisture retention |
| | | Texture improvement |
| | | Enhances fiber content |
| | | Increase yield and hence reduce production costs |
| | Agar | Syneresis control |
| | | Emulsifier |
| | | Adds texture |
| | | Reduces sugar bloom |
| | | Enhances fiber content |
| | | Increase yield and hence reduces production costs |

***Table 3:*** *Potential applications of alginate, agar and carrageenans in diverse food products.*
*Source: Adapted from Reference [4].*

| Polysaccharides | Some major seaweed sources | Some major bioactivities |
|---|---|---|
| Agar | *Gracilaria* spp.<br>*Gelidium* spp.<br>*Hypnea* spp. | Anti-HIV, anti-inflammatory activities, use as laxative and for treatment of constipation. |
| Alginic acid (alginate) | *Macrocystis* spp.<br>*Laminaria* spp.<br>*Ascophyllum* spp.<br>*Sargassum* spp.<br>*Ascophyllum* spp. | Antibacterial, pre-biotic, hypo-cholesterolemic, anti-hypertensive, anti-ulcer activities. Functions as dietary fiber, potentials to treat diarrhoea, constipation. Useful in wound dressings, drug delivery. scaffold for reconstructive processes, etc. |
| Carrageenans | *Euchema cottoni*<br>*E. spinosum*<br>*Gigartina stellate Chondrus* spp | Anticoagulant, control of ulcer, immunomodulation activities. Stimulates collagen biosynthesis hence potential use in skin care.<br>Potential to inhibits herpes, HIV |
| Fucoidans | *Fucus serratus*<br>*Fucus vesiculosus*<br>*Ascophyllum nodosum*<br>*Laminaria* spp.<br>*Ascophyllum* spp. | Anticoagulant, antitumor, anti-inflammatory, antioxidant, immune-stimulant, antiviral, antibacterial, heparin-like, hypoglycemic, hypolidemic activities.<br><br>May ameliorate chronic renal failure |
| Laminarin | *Laminaria japonica*<br>*L. digitate*<br>*L. hyperborean*<br>*Fucus vesiculosus*<br>*Ascophyllum nodosum* | Antimicrobial, anticoagulant, hypo-cholesteromic, antihypertensive, immune-stimulating, cytotoxic and, wound healing and prebiotic activities. Potential use as surgical dusting powder. |
| Furcellaran | *Furcellaria lumbricialis*<br>*F. fastigiata* | Limited biomedical applications |
| Ulvan | *Ulva* spp. such as *U. conglobate*, *U. lactuca* and *U. rigida* | Anticoagulant activity like heparin,<br><br>Potential apoptotic/programmed cell death activity,<br><br>Antitumor activity, (potential to treat gastric ulcer), antiviral and immunomodulating activities |
| Porphyran | *Palmaria palmate*<br>*P. umbilicalis*<br>*P. palmate*<br>*P. umbilicalis* | Potential to induce apoptotic/programmed cell death, possible anti-tumour activity, use as dietary fiber |

***Table 4:*** *Polysaccharides from some major seaweed species, their bioactivities and medicinal uses.*

Seaweeds and also their polysaccharides enjoy established commercial markets [74]. Seaweed products have a long tradition in Asian cuisine. Nutritionally, they are well recognized for their nutrients such as fiber, protein and minerals [75-77]. Many seaweed species have been approved by the Codex Standards of the Food and Agriculture Organization (FAO) of the United Nations, Rome, as sources of commercially important polysaccharides (hydrocolloids). These include Danish agar (*Furcellaria fastigiata*), Eucheuman (*Eucheuma* spp.), Hypnean (*Hypnea* spp.), Iridophycan (*Iridaea* spp.) and Irish moss (*Chondrus* spp.) [2,9]. Agar, alginate and carrageenan are established food additives, approved by the Codex Alimentarius Commission (CAC) of the FAO, the US Food and Drug Administration (FDA) and the

European Council (EC). Alginic acid, its sodium, potassium, ammonium and calcium salts and propylene glycol alginate (PGA) have been approved by the EC [74]. Carrageenan is a permitted food additive. Whereas carrageenan *per se* is safe, its degradation products may cause concern due to their toxicological properties. Molecular weight determination of carrageenan prior to their use in foods can ensure that degraded products are not used [29].

## Conclusion

The global market for hydrocolloids including seaweed polysaccharides is expected to rise from 5.5 billion in 2017 to nearly $7.0 billion by 2022. The market values for alginate, agar and carrageenans in the year 2016 were 346.9, 346.9 and 624.5 million US $ (www.bccresearch.com). Commercially important preparations of seaweed include processed Euchema Seaweed (PES), semi-refined carrageenan (SRC), Alternatively Refined Carrageenan (ARC), all of which are valuable food additives. 'Modifilan', is a patented commercial extract of *Laminaria* spp., containing significant amounts of organic iodine, fucoxanthine, alginate, fucoidan and laminarin. Sulfated polysaccharides, particularly fucoidans, have shown significant potentials for uses in pharmaceutical field [16]. Fucoidan from the seaweed, *F. vesiculosus,* is available from Sigma-Aldrich Chemical Company, St. Louis, MO, U.S. [25]. Oligosaccharides such as fructo-oligosaccharides and galacto-oligosaccharides are commercially used, mostly as food additives [78]. It is likely that applications of seaweed polysaccharides, their derivatives and degradation products in food, pharmaceutical and other industries will increase in the coming years supported by further understanding of their functional properties and bioactivities together with developments in down-stream processing for their isolations.

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**Volume 14 Issue 2 February 2019**