MARINE POLYSACCHARIDES & THEIR OLIGOMERS: FUTURE PROSPECTS AS NUTRACEUTICALS

Renuka V*., Elavarasan K., Lekshmi R.G.Kumar., C.S. Tejpal & Anas, K.K

ICAR- Central Institute of Fisheries Technology (CIFT), Kochi-29

*renuka.v@icar.gov.in

Introduction

The marine realm covers 70 % of the earth's surface making the oceans the largest ecosystem on earth, which may contain over 80 % of the world's plant and animal species. The ocean is known as a vast and renewable source of natural substances. Among marine biomaterials, polysaccharides have received increasing attention in recent years because they are abundant, cheap, biocompatible, and biodegradable facilitating their biomedical applications in drug delivery systems, tissue engineering, cancer therapy, or wound healing. Functional foods and nutraceuticals provide an opportunity to improve human health, reduce healthcare costs and support economic development in rural communities. In this chapter, we overviewed the nutraceutical application of marine polysaccharides including carrageenan, alginate, agar, fucoidan, agarose from seaweeds and chitosan from crustacea.

Seaweed Polysaccharides

Marine polysaccharides from seaweeds are an integral part of a globally thriving marine-bio industry. Seaweeds are the most abundant source of polysaccharides as alginate, agar, fucoidan, agarose, as well as carrageenan. The content of polysaccharide present in seaweed is high, accounting for more than 50% of the dry weight.

Carrageenan

Carrageenan an anionic sulfated polysaccharides that are extracted from the red algae Rhodophyceae family. The main components of the cell walls of red seaweed are carrageenans and represent between 30% and 75% of the algal dry weight. These polysaccharides are considered the most widely studied red algae polysaccharides.

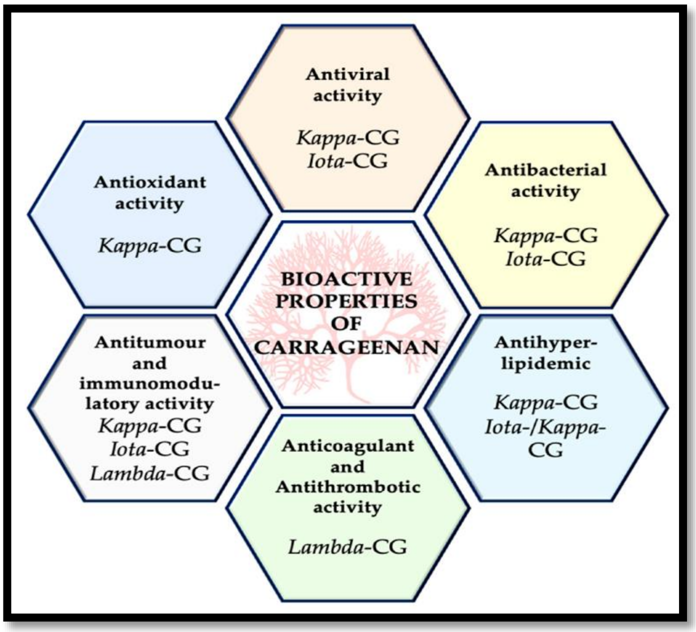
Carrageenan is composed of alternate units of β-d-galactose and 3,6-anhydro-α-d-galactose, linked by α-(1,3) and β-(1,4) glycosidic unions. Carrageenans were used as a thickening agent in the food industry, and due to their gelling, emulsifying, and stabilizing properties. It is recognised as "Generally Recognized as Safe" (GRAS) by the Food and Drug Administration (FDA) since 1973 (FDA SCOGS (Select Committee on GRAS Substances). Carrageenan (E-407) and semi-refined carrageenan (E407a) have been approved by the European Food Safety Authority as food additives. The toxicological aspects of Carrageenans have been thoroughly evaluated and they have been established to have minimal or no adverse physiological effects. Carrageenan has no nutritional value. It have been used to increase the texture quality of cottage cheese, to maintain the viscosity of pudding and dairy products, and as binders and stabilizers in the production of patties, sausages, hamburgers and meat-processing.

Carrageenans are of different forms and they can be identified in terms of the chemical structure they have. There are three major types of carrageenan, which include iota-carrageenan (i-carrageenan), kappa-carrageenan (κ-carrageenan), and lambda-carrageenan (λ-carrageenan).

Most carageenans are extracted from *Kappaphycus alvarezii* and *Eucheuma denticulatum.* Kappa-carageenan is mostly extracted from *Kappaphycus alvarezii*, commercially known as *Eucheuma cottonii*, while iota-carageenan is predominantly produced from *Eucheuma denticulatum*, also known as *Eucheuma spinosum*.

The manufacturing of CG consists of extraction, purification, concentration, precipitation, filtration, and drying, although the process may vary according to the family of red algae used to extract the sulphated polysaccharide. Carrageenans have shown potential bioactive qualities, including antiviral, antibacterial, antihyperlipidemic, anticoagulant, antioxidant, antitumor, and immunomodulatory properties.

Due to their biocompatibility, high molecular weight, high viscosity, and gelling capacity, these polymers have gained great importance in recent decades not only in the food industry but also in medical, pharmaceutical, and biotechnological research.



Bioactive properties of the different types of carrageenans used in biomedical applications *(Adopted from Quito et al., 2020).*

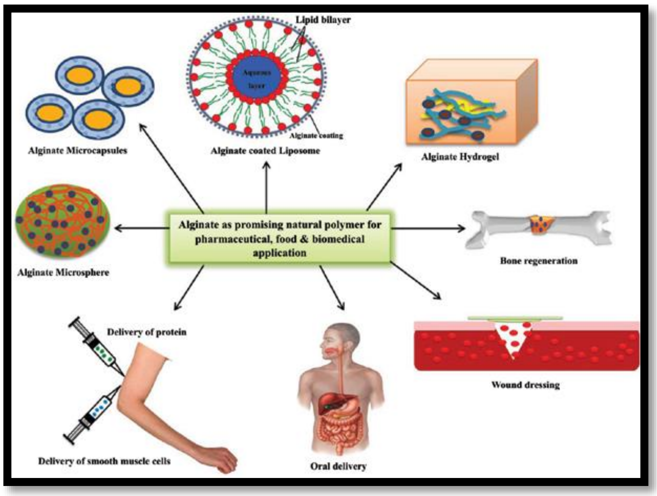
Alginate & its oligosaccharides

Alginate is an anionic polysaccharides and commonly available in cell wall of seaweed. It is a major constituent of the brown algae (mainly sargassum algae and kelp) cell wall represent between 30% and 75% of the algal dry weight. It composed of β-Dmannuronic acid (M) and α-L-guluronic acid (G) linked by 1,4-glycosidic bonds. It is not only a biopolymer but also a polyelectrolyte that is considered to be non-toxic, biocompatible, biodegradable, and non-immunogenic. Alginate is mainly extracted from the *Laminaria hyperborea, Laminaria digitata, Laminaria japonica, Ascophyllum nodosum, and Macrocystis pyrifera*. Alginate is mainly extracted based on its high solubility in alkaline solution and low solubility in water. Alginate extracted from different species of seaweeds differ in G and M contents, which are present in the polymer chain as GG, MM and MG/GM blocks of various proportions, resulting in the differences in the physical properties of the respective alginate products. The content of G in Sargassum is higher, while the content of M in kelp is higher. The M/G ratio in alginate is not fixed. Even for the same seaweed, the proportion will change with different growth years, picking seasons and locations.

Alginate is an established food ingredient widely used in the production of functional food products. It forms a viscous gum with water and having capacity to absorb water around 200–300 times of its own weight. Alginate is widely used in industry because of its ability to gel with calcium ions; however, this property is also strongly influenced by its uronic acid composition. Because of the thickening, gelling and film forming properties, alginate is widely used in the food and drink industries, functioning as one of the most important food ingredient.

The applications of alginate are based on three main properties. The first is its ability to thicken the resulting solution when dissolved in aqueous solutions, i.e., its ability to increase the viscosity of aqueous solutions. The second is its ability to gel when a calcium salt is added to a solution of sodium alginate in water, with no heat required to form the heat stable gel, which is different to carrageenan and agar gels where the water must be heated to about 60°C and 80°C respectively for dissolution to take place, with detrimental effect on the bioactive components involved. The third property of alginate is the ability to form films of sodium or calcium alginate and fibers of calcium alginates. In addition, its unique structure is also related to some biological functions that are highly beneficial to human health.

Alginate oligosaccharides are compounds of low molecular weight (MW) composed of 2–10 monosaccharide units. They may naturally occur in foods or may be prepared by the synthesis of disaccharides or by the hydrolysis of polysaccharides. Generally, sodium alginate is used in the food and beverage industry as a stabilizer, thickener in the preparation of drinks, ice cream, and jelly, as well as an encapsulation material of yeast cells in ethanol production. Unfortunately, sodium alginate does not possess biological activity which could expand its functional applications. Sodium alginate oligosaccharides formed during the depolymerization process generally do not have the ability to form gels; however, they are characterized by other important biological activities.



Application of alginate in pharmaceutical and biomedical application

Fucoidan

Fucoidan is a long chain sulfated polysaccharide found in various species of brown algae. Fucoidan occurs in the cell walls of the seaweed plant and serves to protect it from external stresses. In general, the polysaccharide backbones of fucoidan are classified into two groups. One group (type I) contains repeated (1–3)-α-L-fucopyranose residues, whereas the second group (type II) includes alternating and repeated (1–3)- and (1–4)-α-L-fucopyranose (Wu et al. 2016). Meanwhile, these two backbones can also carry other monosaccharides such as uronic acid, galactose, mannose and xylose (Álvarez-Viñas et al. 2019). Therefore, the structure of fucoidan is extremely complicated since the locations of these monosaccharides in various kinds of seaweed species are uncertain. Commercially available fucoidan is commonly extracted from the seaweed species *Fucus vesiculosus, Cladosiphon okamuranus, Laminaria japonica* and *Undaria pinnatifida.*

Fucoidan is a unique polysaccharide which has not only strong negative charge but also extensive pharmacological activity and favorable safety. Fucoidan has many properties including anticoagulant, antibacterial, anti-inflammatory, antioxidative, antiviral and antitumor activities, which primarily depend on molecular weight, monosaccharide compositions, sulfate groups and other substituents. Torres et al. (2020) presented that high molecular weight could reduce the antitumoral properties of fucoidan. Additionally, depending on the versatility of its chemical structure, fucoidan is an appropriate carrier with excellent properties. The biological activities of fucoidan are closely related to several intrinsic factors including their molecular weight, monosaccharide composition and content of sulfate group (Ashayerizadeh et al., 2020; Liu et al., 2020).

The primary properties of fucoidan are ionic crosslinking and solubility. Although fucoidan has hygroscopic properties, it does not form highly viscous solutions. Therefore, fucoidan is not employed as a thickener or gelling agent in the food industry like other polysaccharides. The water-soluble sulfated fucoidan facilitates the construction of delivery systems based on fucoidan and other positively charged molecules. The fucoidan-based food grade delivery system has various advantages, such as high loading capacity, nontoxicity, controlled release and protection of bioactive ingredients from degradation in the gastrointestinal tract.

Laminarin & its oligosaccharides

Laminarin is a polysaccharide (dietary fibre), rich in marine brown seaweed. Derived low molecular weight polysaccharides exhibit various biofunctional properties including antitumor, anti-apoptotic, anti-inflammatory, antiobese and antioxidant activity. Among the seaweed polysaccharides, laminarin is an important non-toxic and biodegradable polysaccharide extracted from the cell-wall storage of marine brown algae. It is found as one of the major components, along with alginate and fucoidan. Structurally, laminarin is made up of glucose monomers joined together by (1, 3) linked β-glucans and (1, 6) linked glycosidic bonds. Laminarin has two forms of polymeric chains, G-chains with glucose at the end and M-chains with mannitol at the reducing terminal. Dietary fibres comprise of plant polysaccharides that are resistant to digestion in the upper gastrointestinal tract and absorption in the human small intestine but undergo partial or complete fermentation in the colon. Laminarin is unaffected by enzymatic hydrolysis in the upper gastrointestinal tract. Even though the usage of laminarin is limited to food components, few reports have shown its biological importance to treat metabolic complications. In the recent past, it has been informed that saccharides obtained from laminarin had enhanced biological activities.

Chitosan & its oligosaccharides

Chitin

Chitin is the second most important natural polymer in the world. The main sources exploited are two marine crustaceans, shrimp and crabs. Chitin and its derivatives is the major by product from crustacean processing. Chitin or poly (β-(1→4)-*N*-acetyl-D-glucosamine) is a natural polysaccharide. This biopolymer is synthesized by enormous number of living organisms and it belongs to the most abundant natural polymers, after cellulose. In the native state, chitin occurs as ordered crystalline microfibrils which form structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast.

Chitosan

Chitosan is the most important derivative of chitin. The term chitosan usually refers to a family of polymers obtained after chitin deacetylation to varying degrees. In fact, the acetylation degree, which reflects the balance between the N-acetyl glucosamine and D-glucosamine residues, differentiates chitin from chitosan. When the Degree of acetylation is lower than 50%, the product is named chitosan and becomes soluble in acidic aqueous solutions. Chitin can be converted to chitosan by enzymatic preparations or chemical process. Chemical methods are used extensively for commercial purpose of chitosan preparation because of their low cost and suitability to mass production.

Chitin and chitosan offer a wide range of application from the agriculture to pharmacy industry due to its specific properties like bioactivity, biodegradability, chelation ability, absorption capacity and film forming ability. Although the chitin and chitosan are known to have very interesting physicochemical, functional and biological properties in many areas, their molecular weight and their solubility property restrict their usage. Chitosan, which is soluble in acidic aqueous media, is used in many applications (food, cosmetics, biomedical and pharmaceutical applications). Unfortunately, all chitin and chitosan are not applicable in all sectors owing to its high molecular mass, high viscosity and, thus, low absorption for in vivo applications. The effectiveness of chitosan in various applications appears to be dependent on the degrees of acetylation. Recent studies on chitosan derivatives like Water soluble chitosan, chitooligosaccharides have drawn considerable attention, since the products obtained have been easily water soluble and also possess versatile.

The depolymerised form of chitosans is called as chitosan oligomers or chitooligomers, or chitooligosaccharide (COS). COS has been paid great interest in pharmaceutical and medicinal applications due to their high solubility and non-toxicity. Generally, hydrolysis of chitosan yields the COS and it can be physical, chemical or enzymatic hydrolysis. All these methods are aimed to produce COS with lower Mw and different DD and depolymerisation. Looking back through past research, several technological approaches have been taken to prepare COSs, and enzymatic approaches are favorable due to their environmentally friendly methods, safety, and a lack of toxicity. Similar to chitosan, COSs can also be considered potential nutraceuticals due to their versatile biological activities, water-soluble properties, and absorption properties in the intestine. COSs have significant applications in the development of functional foods.

Low-molecular-weight water-soluble chitosan or COSs are known to have many desirable biological activities such as antifungal activity, antibacterial activity, antitumor activity, immuno-enhancing effects, and protective effects against infection. The molecular weight or chain length, which is generally referred to as the degree of polymerization (DP) and the degree of acetylation (DA) are considered as principal characteristics of COSs related to their biological activities.

Conclusion

Nutraceuticals and functional food industries have grown significantly in the last few decades. Nutraceutical products provide people with safe and healthy lifestyle. Marine polysaccharide based products have been used as a promising source of bioactive molecules to prevent and cure various diseases for centuries.

Reference

1. Álvarez-Viñas, M., Flórez-Fernández, N., González-Muñoz, M. J., & Domínguez, H. (2019). Influence of molecular weight on the properties of Sargassum muticum fucoidan. Algal research, 38, 101393.
2. Ashayerizadeh, O., Dastar, B., & Pourashouri, P. (2020). Study of antioxidant and antibacterial activities of depolymerized fucoidans extracted from Sargassum tenerrimum. *International journal of biological macromolecules*, *151*, 1259-1266.
3. Fujihara, M., & Nagumo, T. (1992). The effect of the content of D-mannuronic acid and L-guluronic acid blocks in alginates on antitumor activity. *Carbohydrate research*, *224*, 343-347.
4. Huang, Y. C., and T. H. Kuo. 2016. O-carboxymethyl chitosan/fucoidan nanoparticles increase cellular curcumin uptake. Food Hydrocolloids 53:261–9. doi: 10.1016/j.foodhyd.2015.02.006.
5. Liu, Q., Chen, J., Qin, Y., Jiang, B., & Zhang, T. (2020). Zein/fucoidan-based composite nanoparticles for the encapsulation of pterostilbene: Preparation, characterization, physicochemical stability, and formation mechanism. *International journal of biological macromolecules*, *158*, 461-470.
6. Naidoo, D., Kar, P., Roy, A., Mutanda, T., Bwapwa, J., Sen, A., & Anandraj, A. (2021). Structural Insight into the Binding of Cyanovirin-N with the Spike Glycoprotein, Mpro and PLpro of SARS-CoV-2: Protein–Protein Interactions, Dynamics Simulations and Free Energy Calculations. *Molecules*, *26*(17), 5114.
7. Pereira, L., & Critchley, A. T. (2020). The COVID 19 novel coronavirus pandemic 2020: seaweeds to the rescue? Why does substantial, supporting research about the antiviral properties of seaweed polysaccharides seem to go unrecognized by the pharmaceutical community in these desperate times?. *Journal of Applied Phycology*, *32*(3), 1875-1877.
8. Silva, V. A. J., Andrade, P. L., Silva, M. P. C., Valladares, L. D. L. S., & Aguiar, J. A. (2013). Synthesis and characterization of Fe3O4 nanoparticles coated with fucan polysaccharides. *Journal of Magnetism and Magnetic Materials*, *343*, 138-143.

9. Torres, M. D., Flórez-Fernández, N., Simón-Vázquez, R., Giménez-Abián, J. F., Díaz, J. F., González-Fernández, Á., & Domínguez, H. (2020). Fucoidans: The importance of processing on their anti-tumoral properties. *Algal Research*, *45*, 101748.
10. Tran, P. H., Duan, W., & Tran, T. T. (2020). Fucoidan-based nanostructures: A focus on its combination with chitosan and the surface functionalization of metallic nanoparticles for drug delivery. *International Journal of Pharmaceutics*, *575*, 118956.
11. Wu, L., Sun, J., Su, X., Yu, Q., Yu, Q., & Zhang, P. (2016). A review about the development of fucoidan in antitumor activity: Progress and challenges. Carbohydrate Polymers, 154, 96-111.