Seaweed and Their Uses

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From time immemorial man has been utilizing seaweeds, as food, medicine and fertilizer. In a Chinese book of poetry written at the time of Confucius, there is a poem that mentions a house wife cooking seaweeds. These plants have been considered to have medicinal value in the orient. Agar is a phycocolloid (algal colloid) that can be extracted by hot water. Three major phycocolloids are agar, alginates and carrageenan. They are extracted from brown seaweeds and red seaweeds. In Japan agar is believed to be discovered by Minoya Tarozaenon in 1658 and a monument was erected in Shinuizumura to commemorate the first time manufacturing of agar. Originally it was made and sold as an extract in solution (hot or in gel form in cold). The word agar has a Malaysian origin. Agar is the most accepted term although in French and Portuguese speaking centers it is also called *'Gelora'*.

Sources of agar

Different seaweeds used as the raw material in agar production have given rise to products with varying qualities although they can all be included in the general definition of agar. For this reason, when agar is mentioned, it is customory to indicate the original raw material as this can affect its application. Hence there are nomenclature like Gelidium agar, Gracilaria agar, Pterceladia agar etc. To describe the product more accurately, it is usual to mention the original seaweeds like Gracilaria agar from Chile, Gracilaria agar from Argentina, Gelidium agar from Spain, and Gelidium agar from Mexico etc. (Selby, 1969). Originally Gelidium agar constituted what we consider as genuine agar. Currently agar industry identifies uses for following seaweeds:

1. Different species of Gelidium harvested mainly in Spain, Portugal, Morocco, Japan, Korea, Mexico, France, USA, China, Chile and South Africa.
2. Different species of Gracilaria harvested in Chile, Argentina, South Africa, Japan, Brazil, Peru, Indonesia, Philippines, China, India and Sri Lanka.
3. *Pterocladia capillace* from Azores (Portugal) and *Petrocladia lucida* from New Zealand.
4. Gelidiella from Egypt, Madagascar and India.

Industrial harvesting technique for agarophytes vary depending up on circumstances, but they can be classified as follows:

a) Collection of seaweeds from shores
b) Collection of seaweeds by cutting them out from their beds
c) Cultivation or farming

In India seaweeds washed to the shores are collected. In some countries these seaweeds are called agaroze or beach wash. These are dead seaweeds that after completing their biological cycle are separated by seasonal storms. They are gathered by hand or by mechanical means from the coast. Collecting seaweeds by cutting or rooting them out from their beds is done with rakes or grabs handled from boats or scuba divers who operate from boats using compressed air bottles or more frequently by air compressor on the boats connected to the diver by a hose. Gelidium is usually obtained from rocky beds whereas Gracilaria occurs on sand beds.

Farming of seaweeds has become an industry in several countries like China, Taiwan, Philippines, Indonesia and Malaysia. Today seaweeds are extensively cultured as there is a great demand for agar and carageenan (Glicksman, 1981; 1983). Actually agar is a gel forming substance soluble in hot water and requiring 1% solution to get a gel on cooling.

Chemical structure Early studies of agar showed that it contained galactose, 3,6 anhydrogalactose and inorganic sulphate bonded to the carbohydrate as reported by Hands and Peats (1938). Agar is considered to be consisting of two fractions, agarose and agaropectin.

Agarose is found to be neutral, long chain molecule formed by β-D galacto pyronose residues connected through C_1 and C_3 with 3, 6 anhydro L-galactose residues connected through C_2 and C_4. Both residues are repeated alternately (Hjertan, 1971). The links monomers have different resistance to chemical and enzymatic hydrolysis. 1,3 links are more hydrolysed by enzymes (of *Pseudomonas atlantica*) and new agarobiose results. Nevertheless, 1,4 B links make the polysaccaharide chain particularly compact and resistant to breakage as is found in the peptidoglycan of bacteria. The molecular weight assigned to non-degradable agarose is approximately 1,20,000. This has been determined by sedimentation measurements and it represents 400 agarobiose (800 hexose) linked together (Roison, 1965).

Polar residues such as pyruvic acids and sulphuric acids are also found in small quantities. They may come from the small amount of agaropectin left in the agarose after its preparation, but it is opined that sulphate and pyruvate group remain linked in small quantities to the agarose structure depending upon the seaweed used in the agar production. There are number of methods adopted for the production of agar (Yaphe, 1984; Lahaya *et. al.*, 1986). Agaropectin have low gelling power in water, they are supposed to be formed by and alternating groups of D-galactose and L-galactose and they contain all polar groups existing in agar. The basic structure of agaropectin consist of alternatively D-galactose and L-galactose. D-galactose can be substituted by D-galactose-4 sulphate by 4.6 (1 carboxyethylidine)-galactose in certain terminal chain position or even by D-galactose 2,6 sulphate (Hirase, 1957).

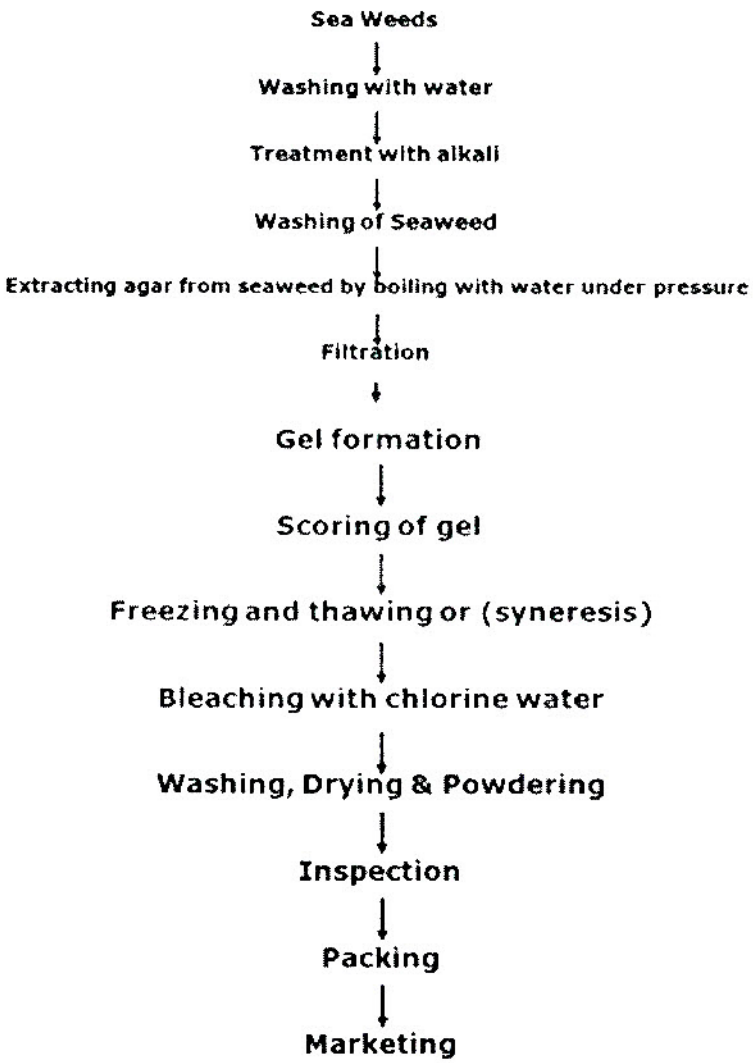
Manufacture of agar Important agarophytees available in Indian coastal waters as per the ICAR-CMFRI Bulletin No. 41 are *Gelidiella acerosa, G. indica, G. pusillum, G. edulis, G. verrucosa, G. crassa, G. vericosa* and *G. coticatta*. There are other species of seaweeds (Agrophytes) available in Indian coastal waters which are not harvested at commercial level. The harvested or collected seaweeds should be thoroughly washed in sea water to remove sand and other foreign material and then sundried. If the seaweeds are to be kept for a long time, they should be treated with formalin and then sundried. The fresh seaweeds are soaked in 2% formalin in wooden or cement lackes for two hours and then sundried. The percentage of moisture and purity decide the cost of seaweed at the time of marketing. When dry yield is about 15% of fresh material, commercial dried material is 60% pure with moisture content of 22%.

Gracilaria edulis is widely exploited for industrial purposes in India. Collection is possible throughout the year around the islands in the Gulf of Mannar, Tamil Nadu. *Gracilaria crassa* has been collected from Vedali and Kilakarai in Tamil Nadu. It grows in shallow areas attached to pebbles and stones. Collection is done by hand picking. *Gelidella acerosa* is collected very rarely and it fetches very good price. Harvesting and collection of seaweeds are done in Indian waters not by design; but by accidental collection. Culturing of seaweeds especially Agarophytes are not popular in India because, the coastal waters are turbulent. The yield of agar and its gel strength vary from species to species and also depends on the method of extraction of agar. Processing conditions play a significant role in the quantity of the end product. Cottage industrial method for the extraction of agar is as follows: Sun bleached seaweeds are washed in freshwater and wet grinded in a stone mortar to remove the impurities and other foreign materials. The seaweed is heated to 90 °C and the pulp is bleached and extracted in potable water. Agar gel obtained is filtered using a cloth filter. Gel is allowed to cool at room temperature and frozen in the cold storage. It is cut into stripes and dried in the sun on plastic net frames. In this method, the impurities are removed from the seaweeds before the extraction and not from the gel. The bleaching process may reduce the cost of production of agar since large scale equipment are not used in freezing. Agar obtained by this dilute acid method may not be in good quality. Various workers had reported the extraction of agar in India by treatment with dilute acids (Bose *et. al.*, 1943; Thivy, 1958). The problem with this method is that the quality of agar, especially gel strength is very low. Mathew *et. al.* (1993) used a mild alkali treatment of seaweeds prior to extraction of agar by usual extraction method. In this method washed seaweeds were treated with 0.5% sodium carbonate solution for a few minutes at a temperature of 90-95 °C. By this treatment, the quality of agar obtained is better than the conventional method and gel strength is also high.

Manufacturing process Agar manufacturing process has undergone modernization since the early stages of freezing methods. For the process, the following criteria should be taken into consideration. Firstly, it is necessary to obtain an extract from agarophyte which contain the largest possible quality of existing agar. Secondly the agar content should have the best possible characteristics to satisfy the standards. For expected standard for the product, especially in gel strength, the following basic points are considered for achieving the required quality: Seaweed treatment prior to extraction, controling the molecular weight distribution during extraction, removal of undesired

products, need to work with large volume of the extract and economics of dehydrating the dilute extracts.

A Flow chart of Agar Production in small scale Industrial Level.



Syneresis

Syneresis is a process in which a gel contracts on standing and exudes liquid. In this process, pressure is applied to exude liquid from the gel. Initially long synerisis period are required ie. longer than 24 hrs that would start with a gradual and slow increase in pressure by placing successively and at a pre-fixed rate, stone blocks on top of the gel container. The agar gel is wrapped in canvas cloths and placed in a series of steel boxes fitted between fixed and movable heads of vertical hydraulic press. This is followed by hydraulic pressing until the product is consistent enough to with-stand extrusion. Now a days the manufacturers designs their own equipment and carries out synersis. Starting with 1% agar extract, synerisis increases the concentration to a maximum of 25%. A food-grade agar usually has a moisture content of less than 18%, ash below 5%, low gel strength and bacterial count below 100000 per gram. *E. coli* and Salmonella must be absent and other pathogenic bacteria must be specified. Usually the lead content is specified as less than 5 ppm and arsenic less than 3 ppm.

Specification for Food Grade Agar

Characteristics	Requirements
Colour	White, pale yellow
Odour	Odorless
Taste	Mucilaginous
Solubility	Soluble in boiling water
Moisture %	20
Total ash % by weight maximum	6.5
Acid insoluble ash % by weight maximum	1.0
Insoluble matter % by weight	1.0
Arsenic (As) as mg/ Kg maximum	3.0
Lead (Pb) as mg/Kg maximum	1.0

Specifications for Bacteriological Grade Agar (IS 6850-1973)

Characteristics	Requirements
Colour	White, pale yellow
Odour	Odorless
Taste	Mucilaginous
Solubility	Soluble in boiling water
Moisture % by weight on drying at 105 °C	20
Total ash % by weight maximum	6.5
Gelatin	To pass the test
Acid insoluble ash % by weight maximum	1.0
Insoluble matter % by weight	1.0
Arsenic (As) as mg/ Kg maximum	3.0
Lead (Pb) as mg/Kg maximum	1.0

Bacteriological agar The use of agar in bacteriology is one of the most important applications and requires a strict chemical control as well as the absence of hemplytic substances and what is more important and difficult is the absence of any bacteriological inhibitors. It's use in bacteriology is based on special properties like gelling temperature of 32-36 °C, melting temperature of 85-86 °C, lack of hydrolysis by bacterial enzymes and it should be free from bacterial inhibitors.

The most important characteristics of agar are the following:

- Gelling power in aqueous environment allows to form gels, which are stronger than any other gel forming agent.
- The simple water solution has the gelling power, one need not add any ions for gelatin like, carrageenan and alginic acid.
- It can be used in a wide range of pH from 5 to 8 and in some cases beyond these limits.
- It withstands thermal treatment very well even above 100 °C, which allows good sterilization.

- An aqueous solution gels between 32-43 °C and does both below 85 °C. This is a unique property of agar.
- Agar gives gels without flavour.
- It assimilates and enhances flavour of product mixed with it and act as a fragrant fixer, permitting long term fixation.
- Its gel has an excellent reversibility allowing it to be repeatedly gelled and melted without losing any of the original properties.
- Transparent gels can be prepared, its refractive index can be enhanced by adding glucose and glycerine.
- The gel is very stable, not causing precipitation and certain precautions are to be taken.

Gel strength

Measurement of gel strength is very important. Various methods are adopted for measuring the gel strength. Nikan–Sui Method is the most commonly used one to measure agar gel strength. This method is based on measuring the load (g/cm^2) that causes a standard gel to break in 20 seconds. A hot 1.5% solution is poured into a metallic box (6x30 cm, 4.5 cm high) to the 3 ml level leaving it to gel at 20 °C. The breaking load withstood for 20 seconds is measured with an apparatus designed by Kiya Siusakushio, Tokyo, Japan.

Uses of agar

Agar was the first phycocolloid to be used in food industry. FAO/WHO Codex Alimentarius permit the use of agar in human food industry. It is used as gelling agent and in secondary way as a stabilizing agent and for controlling viscosity. It is also used as an additive. As the gelling power is very high, it is used at maximum 1% concentration for viscosity control and as a stabilising agent, the concentration used is 1/100 or less (Chapman, 1970; Glicksman, 1981). In confectionary, it is used to prepare jellies, marshmallows, and candies or candy fillers. Agar is used as thickening and gelling agent in the preparation of marmalade. In bakery, it is used to cover cakes as in icing jelly and yoghurt preparations. It is used to prevent dehydration also. It is also used for fruit jelly preparations. In the meat industry also, agar is used for preparing soft boiled sausage. It is also used in casting moulds in dentistry. Another rare application is the

preparation of feed for insects during their metamorphosis stage. The application of agar as a laxative is well known for years. It is also used in orchid nurseries for long time and, used in the tissue culturing of plants.

Uses of agarose

1. Immuno-diffusion and diffusion technique.
2. Used for electrophoresis of particles carrying electrical charges with direct application for proteins, nucleic acids and polysscharides. It has application in conventional electrophoresis as well as in immuno-electrophoresis or electrofofussing.
3. Chromatographic technique in gel chromatography, ion exchange chromatography and affinity chromatography.
4. In bio-engineering and in microbiology as an excellent base for growing very special cultures in many cases related to oncological research.

Carrageenans

Carrageenans constitute the third most important hydrocolloid after starch and gelatin. They are extracted from the marine plants, carragenophyte and the red seaweeds (Rodophyceae). They serve as a structural function analogues to that of cellulose in land plants. Chemically they are sulphated galactans. Philippines is the largest producer of carragenophyte. Irish moss is also known as carrageenan from the Irish word Carraigeen, meaning Rock Moss. Carrageenan is extracted from *Chondrus crispus* by water (Hum, 1957). Seaweeds which are used for the carrageenann production are *Chondrus crispus*, *C. ocellatus*, *Gigartina stellate*, *G. acicularis*, *G. pistillata*, *G. canaliculata*, *G. chamissoi*, *G. radula*, *Gymnogonrus furcellatus*, *Euchema cottoni*, *E. spinosum*, *E. gelatinae*, *Furcellaria fastgita*, *Hypnea mucifermis* and *Kappaphycus alvarezii*. *K. alwarezii* is cultured in coastal waters of Tamil Nadu for the preparation of carrageenan (Periyasamy *et. al.*, 2017).

Chemical composition

Carrageenans have common features of being linear polysaccacharides with a repeating structure of alternating 1,3 linked β D galactopyrranosyl and 1,4 linked A D galactopyrranosyl units. The 3-linked units occur as the 2 and 4 sulphate, or unsulphated while the 4-linked units occur as the 2 sulphate, 2,6 disulphate or unsul 3,6 anhydride

and 3,6 anhydride 2 sulphate. Sulphation at C3 never occurs. Pyruvate has been reported to be present in the carrageenans from *Gigartina* species. These carageenans are termed 'pi carrageenan'. Methyl group occurs in sulphated polysaccharides from the potassium chloride from Gratelonpieeae family.

In their work on fractionation of carrgeenans from *Chondrus crispus*, Smith and Cook (1953) isolated two fractions which they named Kappa and Lamda carrageenans. Kappa was defined as that fraction which was precipitated by potassium chloride, while Lamda was the fraction which remained in solution. Chemical structure of these fractions revealed that half of the sugar units in Kappa were 3,6 anhydro galactose, while Lamda contained none of this sugar. It is found that small quantities of Mu, Iota, Lamda, Theta and Xi are obtained from some species of carragenophytes. Mu and Nu are believed to be precursors in the biosynthesis of Kappa and Iota, respectively, the transformations being accomplished in the algae by an enzyme 'dekinkase'. Lamda can be at least partially converted to Theta carrageenan by this reaction, but Theta has yet to be identified as occurring naturally.

Extraction process

There are different methods for the extraction of carrageenan from seaweeds. It is dried and baled, and are received at the processing plants from the harvesting location. The raw material is tested for its moisture, sand and salt content. The non-carrageenophytes are also estimated. Weeds may be washed to remove adhering salts, sand stones and marine organisms. Washed weeds are usually blended by selection to achieve desired properties in the extractive. It is then digested with hot water under alkaline conditions to exhaustively extract the carrageenans as reported by Smidsrod (1967). The usually used alkali is either calcium hydroxide or sodium hydroxide. Alkali promotes swelling and maceration of the weed to aid in bringing carrageenan into solution while secondly, when used in high concentrations, it effects cleavage of 6 sulphate groups from the carargeenan to generate 3,6 anhydro D-galactose residues in the polysaccharide chain. These function to enhance the residues in the polysseeaharide chain and the water gel strength and milk reactivity of carrageenans. Maceration is promoted by agitation of the representative paste. Conversion of 6 sulphated moieties to the 3,6 anhydride continues during digestion of paste at temperature near 100 °C. When desired conversion has been achieved, the solution of carrageenan is separated from the weed solids by filtration or by centrifugation followed by filtration.

Concentration of the filtrate by evaporation and adjustment of pH are done prior to the recovery of the carrageenan from solution.

Several methods are used to recover the carrageenans from the solution. Direct drying of the concentrated filtrate on steam heated rolls has been used extensively. Precipitation of carrageenan from the solutions by 2-propanol or alcohol gives high quality product. Precipitation followed by alcohol washes to dehydrate gives high quality coagulam. It is then centrifuged and dried permitting recovery of the residual alcohol. The fibrous carrageenan from the dryer is ground and sieved to specified particle sizes of 80 mesh to 270 mesh. It is then packed in polythene bags.

Semipurified carrgaeenan

The carrageenan industry has received a quantum promotion in marketing with the approval of semi-purified or crude carrageenans by food industries and USFDA in late 1990s. At present, fibre is considered as a vital food ingredient and processed food lacks fibre. Semi-purified carrageenan is nothing but processed seaweed containing all natural fibres. In the Philippines, carrageenan is processed in to three grades: i). PNG Carrageenan, ii). Semi-refined carrageenan, and iii). Refined carrageenan. Preparation of PNG carrageenan is very simple. It is natural carrageenan where the crude nature of seaweed is retained. It contains good quantity of fibres also. In the past US and European industries purchased PNG carrageenan and used to prepare purified sodium and potassium salt and this process was expensive. This has paved the way for further expansion of carrageenophyte market.

Properties of carrageenan

Theoretically a pure carrageenan solution will not form gel even if it is cooled to the freezing temperature. Agar and carrageenan have different uses. Agar is best suited to use in cases where the tendency to form a firm gel is required. Indeed this is the property for which it is best known. Carrageenan is superior for uses which require high viscosity and the concomitant thickening, emulsifying, and suspending properties. However, in some cases carrageenan is used for its gel forming properties.

The chemical reactivity of carrageenan is primarily due to their half ester sulphate groups which are strongly anionic, being comparable to inorganic sulphate in this respect. Commercial carrageenans are available in the form of sodium, potassium and calcium

salts, probably a mixture of all these. Kappa and Iota carrageenan form gels in the presence of potassium or calcium ions where as Lambda carrageenan does not (Pernas, 1976). The functionality of carrageenan in various applications depends largely on their rheological properties. Carrageenan is a linear water soluble polymer typically forming highly viscous aqueous solutions. This is due to its unbranched, linear macromolecular structure and poly electrolyte nature (Morris *et. al.*, 1980). Viscosity depends on concentration, temperature and the presence of other solutes, and the type of carrageenan and its molecular weight. Viscosity increases nearly exponentially with concentration. Salt lowers the viscosity of carrageenan solutions by reducing the electrostatic repulsions among sulphate groups. This behaviour is likewise normal for ionic macromolecules.

Sl. No.	Role of Carrageenan	Uses
1.	Food Clarification liquors/ beverages/chocolates/ milk drinks or shakes	Accelerates and improves
	Ice creams/desserts/gellies	Enhances viscosity, stabilising emulsion
	Processed foods	Prevents ice crystal formation and enhances softness as a binder
2.	Meat industry/beef patty	Retains moisture and enhances size and consumer acceptance
3	Meat preparations	As an extender and emulsifier, control freeze dehydration, fat oxidation and retains ntural texture of meat under frozen storage
4.	Non-food/Pet food	As a binder
5	Canned fish/meat	As a gelling and stabilising agent
6	Tooth paste	As a vehicle for gelpastes and stabiliser
7	Fish feed/prawn feed	As a binder

At low temperature and high enough salt concentration, carrageenan solution may gel with an increase in apparent viscosity. This is particularly true for strongly gel-inducing cations K^+. At high temperature, however, Ca^{2+} lowers viscosity to a greater

extent than does Na^+ or K^+. Commercial carrageenans are available in viscosities ranging from about 5 mpa to 800 mpa when measured at 1.5% concentration at 75 °C. Carrageenan solutions having viscosities less than 100 mpa, have flow properties very close to Newtonian. Carrageenan usually used for water thickening applications are Lambda types or the sodium salt of mixed Lambda and Kappa. Kappa and Iota carrageenas and furcellaran form gels on cooling of their hot solutions in the presence of 1.4 linked 6 sulphated residue in the polymer chain of either Kappa or Iota carrageenan detracts from the strength of their gel. Kappa and furcellaran carrageenan gels are relatively rigid and are subjected to synersis. Iota carrageenan by itself yields compliant gels with very little tendency to undergo synersis. Potassium ion is more effective than calcium ion in inducing gelation of Kappa, the reverse is true for Iota carrageenan (Stancioff and Renn, 1975). All carrageenans have the ability to form gels by cooling a solution of the carrageenans in hot milk. Even Lambda carrageenan which does not gel in water will form a gel at levels of 0.22% or more by weight of the milk. This gelation is ascribed to the formation of carrageenan casein bonds.

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