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Agar-agar extraction, structural properties and applications: A review

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Abstract
Agar is a Japanese agarophyte seaweed that was first brought to the Far East and then to the rest of the world. It is a gel-forming polysaccharide that produces D-galactose when hydrolyzed with acid. The chemical structure of agar is comprised by its components namely agarose and agaropectin. The structure and relationship between these two polysaccharides is quite complex and decides the gelling ability of agar. The chapter discusses about seaweeds utilized for agar extraction as well as the industrial techniques for agar extraction and manufacture along with the filtration and dehydration techniques. The applications of agar in food technology are widespread and well-studied. The chapter presents the latest implications of agar in nanotechnology, pharmacy, and photographic silkscreen printings.

Keywords: Agar, *Gelidium* spp., agarose, agaropectin, gelation

1. Introduction
Agar is a polysaccharide-based hydrocolloid seaweed with significant gelling ability [1]. In the mid era of the 17th century an innkeeper named Tarozaemonminoya in what is now known as Kyoto, Japan by chance discovered agar the documented story goes that a fresh dish made from the seaweed *Gelidium* spp. was served to the high rank officer at his dining during the cold season and the remaining uneaten food pieces in the dish transformed into the white squashy like matter and today we know that squash white matter as agar [2]. In many coastal places the habit of consuming various delicacy prepare from different kinds seaweed extracts extends back to primitive times and is still practiced today.
Prior to World War II Japan was the only producer of agar hydrocolloid with a traditional industrial structure based on small-scale operating industries producing non uniform qualities since the industries were family-oriented with a high level of employees as there was no machinery production. Recently, Japan has shifted to complete modern technology plants for the production of uniform agar production. The times when World War second was at peak, countries with sufficient supply of *Gelidum sesquipedale* that is very much similar to *Gelidium pacificum* took advantages of agar scarcity which was used by Japanese industries. Agar developing and manufacturing enterprises were built by Portuguese and Spanish people after researching about the process of agar development from *Gelidium sesquipedale*. Agar replacements from different seaweed extracts were also made by European countries that had scarcity of agarophyte algae. For maintaining the prime quality of agar, mostly Red Algae *Gelidium amansii* is employed for agar extraction [3].
Frau fanny Eilshemiushesse a German housewife was the first person who suggested her husband to use agar as growth media to sustain bacterial growth for the further study in biotech and microbiology labs as a replacement for gelatin [4]. During the end of 19thcentury agar is used in laboratories significantly less than it is used in food the majority of countries imported agar from Japan while agar is currently produced in a number of countries other than Japan. Araki's groundbreaking work on agars chemical structure revealed that the major component responsible for gelation is agarose after this discovery the use of agar as gelling agent increase in the scientific and research areas [5]. Sincethen, any research work related to agarose gels is directly applied to agar gels as well.
Seaweeds are also known as the "Medical Food of the Twenty-First Century" [6]. Mostly the raw material comes form the natura sea weed beds which are utilized to make agar, alginates, and seaweed liquid fertiliser in India. In the marine states of Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, and Gujarat, there are around 25 agar and 10 algin factories located in various locations. Red algae like *Gelidiella acerasa*, *Gracia riaedulis*, *G. crassa*, *G. faliilera* and

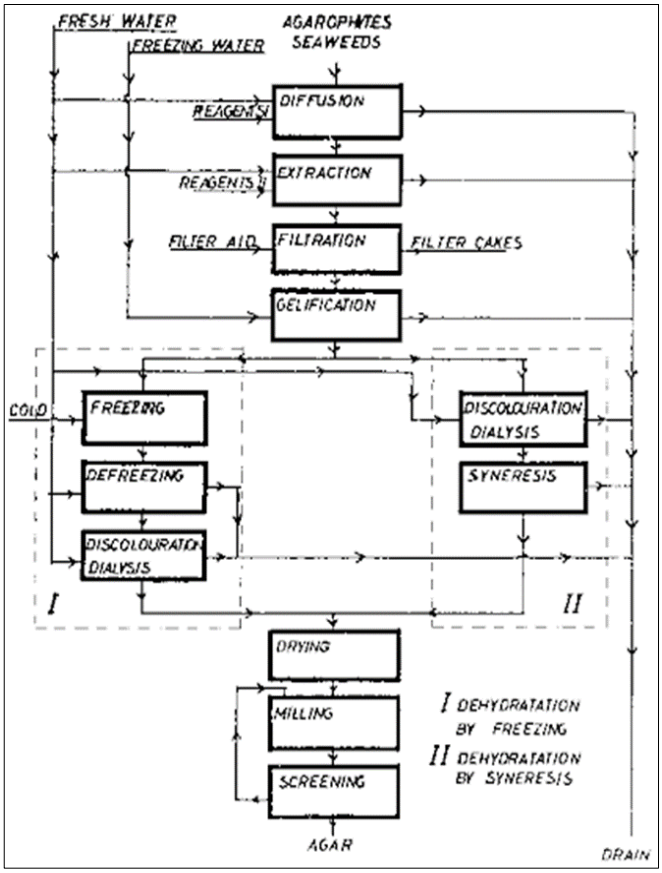
G. vecasa are now used to make agar, whereas brown algae like *Sargassum* spp., *Turbinaria* spp., and *Cystoseira indica* are used to make alginates and liquid seaweed fertilizer. Among these agarophytes, *G. acerosa* produces bacteriological grade agar, whereas *Gracilaria* species produce food quality agar [7].

2. Extraction

2.1 Traditional method of Agar extraction

For agar extract, refined seaweeds must be cooked with additional amount of water at scorching temperatures of more than 100 ºC. Accurate amount of measured acid such 0.02% of sulphuric acid or 0.05% of acetic acid is added in the cooking process to facilitate a proper separation. A number of different agarophytes are cooked in a single pot, which can hold up to 3-8m^2 of components are used in Japanese traditional method.

Industrial agars are made in advanced factories that generate fully standardized agars that fulfill physico-chemical and pathological criteria in line with sanitation maintaining norms. The best plants meet ISO-9000 standards, which provides the guarantee to the markets about all under controlled operations and the exported raw materials and goods can be traced.



Source: Armisen and Galatas (1987) [49].

Fig 1: Agar fabrication diagram.

2.2 Extraction under Pressure

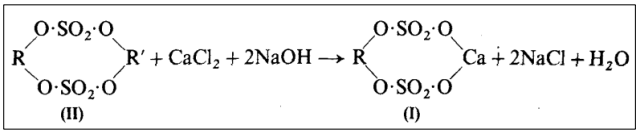
This method works better for firm type seaweed, increases agar production in less amount time compared to other extraction methods. A gauge compulsion of 1-2kg/cm^2 is usually applied for 2-4 hours. Comparative methods for the derivation of hard African Gelidiaceae spp *cartilagineum* revealed that under environmental conditions pressurized cooking extraction yielded more agar than normal acid-cooking extraction. It is necessary to develop unique extraction circumstances and machinery methods in the factory for every different type of seaweed. however, the acid cooking method and pressure method provide good results for agar production, but these both techniques are catastrophic for produced agar. Although agarose is a critical element in agar for gelation, agaropectin does not prevent agar from gelling. To improve the gelling ability, removing the agaropectin is not always beneficial [8].

2.3 Alkali Treatment of Seaweeds

Widespread use of *Gracilaria verrucosa*, gathered in Tokyo bay in the postwar period, aided in the development of procedures of agar hydrogel in all over the world as per the socioeconomic background of the agar development. Kojima and Funaki at the Tokyo Institute of Technology were the first to develop the alkali treatment process for the agar industry independently similarly applied to carrageenan extraction.

To improve the gelling ability of agar family, alkali pretreatment is applied [9-12] Despite the fact that it decreases yield and creates an effluent that, if not treated, might constitute an environmental hazard [13, 14].

The proposed exchange process, together with the partial removal of sulpates, is used to convert the mucilaginous substance similar to agaroid in Graciaria to agar at 85-90°C with the help of alkali treatment.



In Graciaria, R and R' are polysaccharide radicals. *Gelidium spp.* are thought to be rich in (I)-form polysaccharides with significant gelling ability, whereas *Gracilaria spp.* are thought to be rich in (H)- form of polysaccharides without any gelling ability. Within *Gracilaria* plants alkali treatment can produce the substitution product (I). As a result, an aqueous sodium hydroxide solution with a trace amount of ionized calcium is utilized. Pretreatment with alkali produces a better grade agar that is easily soluble in water and leaves very little insoluble debris, even after open boiling [15]. Alkaline pretreatment of the agar extraction method was suggested to aid altering the molecular weight by converting L-galactose sulphate to 3, 6-anhydrogalactose [16].

2.4 Extraction by Polyphosphates

Polyphosphates can also be called as condensed phosphates, are useful for extracting agar from rigid-type agarophytes. Phosphates with a higher degree of condensation have superior agar extractability. Although, use of polyphosphates for making agar leads to ionized iron sequestration in the extract. Demonstration have been put forth regarding agar extractability using Gamma radiation, pretreatment of agar seaweeds and extraction in aqueous ammonia, but none of these methods have been used in industry.

2.5 Freezing-thawing method

The traditional process of natural agar production was used at very first, and it was mostly unchanged until 1939, when American Agar & Co. (USA) began manufacturing agar in industries, in freezing tanks similar to those used to make ice bars. The same approach was used in Japan after WWII, as well as in new facilities erected in Spain, Portugal, and Morocco. After thawing and filtering, the extract from

seaweeds, which generally includes between 1 to 1.2% agar during the process, is sharply concentrated to 10 to 12% agar, which is an increase of about ten times. Gelling type of polysaccharides can easily be separated from non gelling types by freezing followed by thawing or pressing. Some non-gelling polysaccharides have been identified as having an agar [17-20].

2.6 Conventional method of agar extraction

Traditional techniques, such as freezing-thawing, are more costly than syneresis. The dry extract weight after pressing is 20% in agar by syneresis, compared to 11% in freezing. When agar is frozen, the water content doubles, trapping impurities, whereas syneresis removes more soluble contaminants. Syneresis agars have reduced ash content. As a result, conventional methods like as syneresis are commonly utilized in agar extraction.

2.7 Syneresis method

Syneresis method employs the gelling quality of colloids, which allows absorbed water to be detached with the application of a sufficient force. In Japan, the technique was only used on a semi-refined scale for *Gracilaria* agar. To drive the water out of the gel, the extracts were packed in cloths with a tight net and crushed beneath stone blocks. After that, it was crushed using small hydraulic presses to remove any remaining water.

A sophisticated syneresis process to concentrate gelled extracts from all agarophytes in 1964 was developed by a pioneer of Hispanagar named Prona, establishing extraction units in Mexico, France, Chile, and South Africa, as well as modernizing existing ones in Spain, Morocco and Portugal. The company shot to immediate fame as a global agar production leader, which it only had a counterpart like Chile in the late 1980s and thereafter Japan in the last decade. At the same time, in 1971, Okazaki claimed that syneresis method was only conceivable for Gracilaria spp. The technology, which allowed for a semi-automatic procedure, was later developed into a fully automated system that we use currently.

2.8 Agar Photobleaching Process

Having concerns for the health of employees and environment safety, an environment friendly approach was required which led to creation of another procedure for agar extraction called as agar photobleaching. The photolysis of CDOM (colored organic matters) is followed by a decrease in the mixed organic matter's absorption coefficients in the UV and visible spectrum ranges. "Photobleaching" is the word for a decrease in light absorption [21]. *G. lemaneiformis* pigments mostly consist of pigments like chlorophyll a, phycoerythrobilin, and carotenoids, all of which can be referred to as CDOM [22]. In an experiment, the physical and chemical characteristics of alkali-modified agar, native agar, chemically-bleached agar, and photobleached agar, were extracted using various procedures, were examined. In terms of gel strength, gelling temperature, sulphate concentration, and 3,6-anhydro-L-galactose content, the photobleached agar outperformed the rest. Gel strength was the highest of photobleached agar among the different processed agars [23].

2.9 Hot Sol Filtration and Gel Dehydration

In the production of agar, diffusion of the hot sol extracts is just as critical as extraction of agar and dehydration of the gel. The majority of traditional Producers use a filtration technique involving coarsely woven cloth bags. It's a simple filtering strategy, but it's the most effective. To successfully create physically pure agar products, a sophisticated agar factory is consists of an autonomously controlled filter press, filter-aid silicate is commonly utilized.

The historical and industrial importance of freezing agar for dehydration of the hydrogel is significant. 'Bar-style agar' and 'stringy agar' were processed using a combination of natural freezing and weather drying. Sublimation accounts for 48 percent of the water removed from frozen agar gel, ordinary vaporization accounts for 12 percent, and defrosted drips account for 40 percent, according to a field experiment in Nagano. For one firm, the benefit obtained for the sublimation of natural heat energy was almost comparable to the two hundred kgs of fuel oil a day. Defrosted drips are more responsible for the water removal in warmer places quantitatively. Mechanical freezing methods are generally used in industrialized enterprises that create ‘dry agar flakes.'

3. Structural properties

3.1 Physico – chemistry of agar

Agar is made up of a diverse population of molecules with varying physico-chemical characteristics. The agar was considered to have just sulphate semiester group along with a few galactoses’ hydroxyl group in a single unitary structure. Then agaropectin and agarose were the two distinct polysaccharides that were found in the agar later [24].

Gel forming attributes of agar the gel-forming ability and solubility of agar polysaccharides are determined by the relative hydrophobicity of the basic repeating unit, the alternating 1, 3-linked b-D-galactopyranose and 1,4-linked 3,6-anhydro-a-L-galactopyranose or agarobiose, and its substitution by hydrophobic (methoxyl) and polar (sulphate, pyruvate) groups [25]. In 1938, the presence of 3-6 anhydro-L-galactose in the agar molecule was also discovered along with some agarobioses. These 11 agarobioses can be generated in a variety of ways by various agarophytes, depending on the gender and species, as well as genetic features. It is regulated by a number of ecological parameters, including the availability of nutrients, the composition of substrates on which they develop, and characteristics of the environment which are hydrodynamic in nature. However, because agar plants grow gradually over the summer season, the harvesting period is more important for the manufacture of such agarobioses.

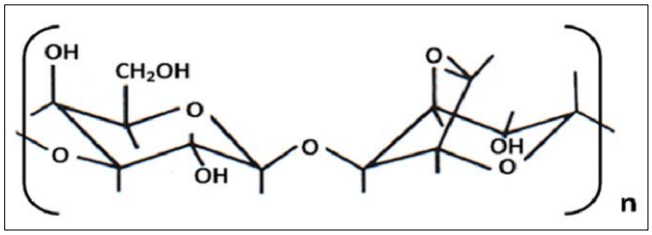
Agaropectin structure is comparatively more multifaceted than agarose. Agaroses are essentially gel-like agar fractions. They have large molecular weights that typically exceed 150,000 Daltons, as well as a low sulphate concentration of less than 0.15 percent. Agaropectine refers to the remaining portions. They have molecular weight of less than twenty thousand Daltons, generally about 14,000 Daltons. Sulfates have a significantly greater concentration, ranging between 5 to 8 percentage in some cases. This level is much less compared to carrageeans, with 24 to 53 percent sulfation even furcellaran, which is the minimum sulfated carrageenan at around 17 percent sulfation.

Agar gels form when a helical structure of agar polysaccharides is possible and the helices may unite. Agar gels are formed when a helical configuration of agar polysaccharides is possible and these helices may aggregate [26]. In the agarose structured conformation, two interwoven left-handed helices with a 3-fold symmetry of pitch 1.90 nm,

axial advance of 0.634 nm, translation between strands of 0.95 nm, and internal cavity of 0.45 nm were investigated. Hydrogen interactions between water molecules and O, galactose, and O, an hydrogalactose stabilize this structure [27]. As a result, single helical clustering might be a step-in agarose gelation.

3.2 Chemistry of agar

Agarobiose has been characterized as a number of variations from the fundamental chemical structure of agarose since its discovery. In *Gelidium amansii* agar, scientists identified a pyruvic acid acetal structurally as 4,6-0-(I-carboxyethylidene)-D-galactose, which is also present in *Gracilaria spp*. agar [28-31] In a study, L-galactose 6-sulfate, a biological precursor of 3,6-anhydrogalactose, has been postulated previously. Scientists have described methylated galactose units such as 6-0-methyl-D-galactose and 4-O-methyl-Lgalactose, as well as L-galactose, methyl-pentose, and xylose. Although the specific position of methyl-pentose in the polymer backbone is unclear, it is known that 4-0-methyl-L-galactose appears as a branch of galactose. Later, the 2-0-methylated anhydro-sugar was discovered in *Rhodomela larix* agar, and it is the main sugar in *Gracilaria eucheumoides* agar, existing with 6-0-methyl-D-galactose and galactose 4-sulfate. Among the most of the commercially available agarose some 6-0- and/or 2-O-methylatedrepeating units are present, indicating that natural methylation of agar is widespread [32].



Source: Ghorbal *et al* (2013) [50]

Fig 2: Chemical structure of agarose

Except for the biological precursor, the majority of these natural chemical changes occur on locations that do not influence the polysaccharide's helical conformation, namely on 0 and 0 of galactose and 0 of 3,6-anhydrogalactose; nonetheless, they may affect helical aggregation and therefore gelation. The creation of a gel is thought to be caused by the aggregation of hexagonal fibres with six double helices [33].

4. Applications

Agars are widely employed in many fields, including the food business, dentistry, bacteriology, and biotechnology [34, 35] In order to predict the high product of agar in each application, it is necessary to better understand the diversity of its commercial goods and its physical and chemical properties. Keep in mind that neither Koch's agar nor today's agarose is necessarily the ideal agar for food applications. 'Many people believe that agar has only one texture: a brittle gel.' This isn't correct. By altering the weed source and processing, it is possible to build a broad range of agar textures, ranging from brittle to highly stretchy.

4.1 Agar in food application

Agar has a variety of uses, the most common of which is as a culinary component, which accounts for 80% of its use about 90% of the extracted agar is utilised in culinary items as a thickener and stabiliser in baked goods, a gelling agent in meats and fish items, and a texture improver in various dairy products such as yoghurt and cheese [36]. Research has been done on agar with its application as a binder. In the research, agar is utilized as a binder for brown rice from corn and red bean flour since it is easier to extract than other binders such as gum or carrageen an. When agar-agar molecules and water are heated in water to create a gel, they move freely [37]. Various attempts to generate medium textured mixed gels between agar-agar gel and gelatin jelly have been made in food processing industry or home cooking because complex food systems have certain textures that are sometimes more effective than any of the individual components alone [38].

Agar can also be used to lower the degree of syneresis, which occurs when water escapes from the grass jelly gel owing to new connections created between the polymers in the grass jelly gel structure. Because it possesses high gel resilience under low pH settings, as well as the ability to bind to the moisture content of the material, agar is ideal for use as a single gelling agent in formulations. Agar can help to decrease syneresis in green grass jelly drinks, with a 20 percent replacement yielding the greatest results. However, because jelly has a strong gelling nature yet breaks readily, a formulation is required to ensure that the texture of the drink is not excessively thick or soft [39].

Agar-agar, which is relatively easy to acquire yet has characteristics comparable to carrageenan, may also be used to make edible film [40, 41]. Aside from that, edible film containing agar may be used as packaging for wrapping jenang cuisine According to the findings of a study on the characterization of a jenang food wrapper manufactured from jackfruit seed flour and agar, the added agar could affect the edible film's thickness, tensile strength and, elongation along with moisture content, and water resistance. This edible film is also classed as environmentally friendly, since it may preserve food goods while still being edible and environmentally beneficial [42].

4.2 Agar in Photographic Silkscreen Printing

As rated by learners, responders, instructors, and when practitioners were pooled together, agar as an addition to an emulsifying agent in photographic silkscreen was quite acceptable in terms of adaptability, long lastingness, and readability. The additive is locally available and simple to manufacture for students who are using the photographic silkscreen technique for business purposes and want to earn profits. According to practitioners, agar-agar is a better option to branded emulsion since it processes faster and produces the same printing outcome [43]. To promote emulsion with better quality while employing low-cost local resources for silkscreen processing, it is more important for the printing industry to develop emulsion with Agar as an additive. Educators should utilise Agar-agar in demonstration units and sessions, especially in photographic printing foundations, because it is safe and natural.

The entrepreneurs can participate in Agar culture, and the technologies dealing for exploiting the indigenous substance as additive can be refined & mass-produced for consumer usage [43].

4.3 Agar in Nanotechnology

Along with its food applications agar is also used in nanoparticle films as a packaging material. The melanin

nanoparticle (MNP) was integrated into the agar film to create a fully functional packaging film, and the findings revealed that the MNP was uniformly disseminated in the agar to produce a composite film. The inclusion of MNP enhanced the composite film's mechanical and water vapour barrier characteristics. UV-blocking effects were seen in the agar/MNP composite films without affecting transparency. In addition, the composite films have a significant level of antioxidant activity. MNP extracted from sepia ink may be utilized to make UV-blocking and antioxidant functional biopolymer composite films for use in food packaging and biomedical applications [44].

According to the findings, agar/lignin/AgNPs nanocomposite films with UV-light barrier characteristics, antimicrobial activities, and enhanced film properties have a great potential for use in active food packaging to ensure food safety and extend shelf life of packed foods [45].

4.4 Pharmaceutical Applications of Agar

Agar-Agar is a very unique naturally occuring polymer that is increasingly favoured over the synthetic polymers and is being explored as a raw material alternative for medicinal applications. It is widely sought after in the pharmaceutical sector due to its remarkable inherent qualities, such as the strength of the gel generated from Agar-Agar.

Agar was used to create an injectable and phase-changeable composite hydrogel for treating cancers with chemo and photothermal treatment. Chemotherapeutics and antibiotics may be loaded into this composite hydrogel and then released. Furthermore, an agar-based nanocomposite film was shown to be effective in preventing Listeria monocytogenes growth. Agar and polysaccharide blends are also gaining popularity in the pharmaceutical industry [46].

Agar-Agar is primarily used in pharmaceuticals as a gelation, stabilization, and thickening agent. Agar-agar is also commonly used for purgative purposes and as a surgical aid. Researchers have worked hard to develop Agar-based products like as composite hydrogels, nanocomposite films, and other materials for application in the pharmacological industry [46].

4.5 Agar Biotechnological Applications

The combined mixtures of agar molecules along with the lowest concentrations of charge leads to the formation of agarose resulting in its best ability of gel formation separated from the complexed molecules called agar present in different amounts of charged groups swapped. Agar's capacity to survive hydrolysis is critical for its application in bacteriology [47]. In the culture media preparation, due to good gel strengthening property and absence of cations with hysteresis a good quality solid microbial culture is prepared. In biotechnology, agarose has a wide range of uses. The innovative applications are likely to drive up demand for best quality agarose in the area of biotechnology growing day by day [47]. Agar derivatives can be employed as dental prosthesis, material shaping, and plant culture tissues in dentistry and biotechnology applications [48].

5. Conclusion

Agar is a gelatinous substance usually obtained from red sea weeds. It is made up of two basic polysaccharides *viz*. agarose and agaropectin. The potential applicants of agar are pharmaceuticals, silkscreen printing, food and packaging industries. Plenty of methods have been identified in order to extract the agar but usage of synersis method have shown promising results among them due to its cost effectiveness and accuracy. In recent past, techniques have been developed to extract the agar with higher solubility with less amount of heat timings and a less temperature range while maintaining acceptable gel strengths. A wider scope can be seen in the recent time for more agar manufacturing process and its applications.

6. References

1. Armisen R, Gaiatas F. Agar. In Handbook of hydrocolloids. Woodhead Publishing, 2009, 82-107.
2. Matsuhashi T, Kanten. In Food Industries, ed. M. Fujimaki *et al*. Koseisyakoseikaku, Tokyo, 1985, 886.
3. Hernández-Carmona G, Freile-Pelegrín Y, Hernández-Garibay E. Conventional and alternative technologies for the extraction of algal polysaccharides. In: Domínguez Hed. Functional Ingredients from Algae for Foods and Nutraceuticals. Woodhead Publishing, Oxford, Philadelphia, 2013, 476-515.
4. Matsuhashi T. Fundamental studies on the manufacture of agar. Dissertation to Tokyo University of Agriculture, 1978.
5. Yaphe W, Duckworth M. The relationship between structures and biological properties of agars. In Proc. Seventh Int. Seaweed Symp., ed. T Nishizawa. University of Tokyo Press, Japan, 1972, 15-22.
6. Khan SI, Satam SB. Seaweed mariculture: Scope and potential in India. Am J Biochem Biotechnol. 2003;1:56-78.
7. Kaliaperumal N, Uthirasivan P. Commercial scale production of agar from thered alga Gracilaria edulis (Gmelin) Silva. Regional Centre 0/ Centr, 1 Marine Fisheries Research Institute Marine Fisheries - 623 no, India, 2001.
8. Nishinari K, Fang Y. Relation between structure and rheological/thermal properties of agar. A mini-review on the effect of alkali treatment and the role of agaropectin. Food Structure. 2017;13:24-34. https://doi.org/10.1016/j.foostr.2016.10.003
9. Mehta GK, Meena R, Prasad K, Ganesan M, Siddhanta AK. Preparation of galactans from *Gracilaria debilis* and *Gracilaria salicornia* (Gracilariales, Rhodophyta) of Indian waters. J Appl. Phycol. 2010;22:623-627.
10. Meena R, Siddhanta AK, Prasad K, Ramavat BK, Eswaran K, Thiruppathi S, *et al*. Preparation, characterization and benchmarking of agarose from *Gracilaria dura* of Indian waters. Carbohydr. Polym. 2007;69:179-188.
11. González-Leija JA, Hernández-Garibay E, Pacheco-Ruíz I, Guardado-Puentes J, Espinoza-Avalos J, López-Vivas JM, *et al*. Optimization of the yield and quality of agar from *Gracilariopsis lemaneiformis* (Gracilariales) from the Gulf of California using an alkaline treatment. J Appl. Phycol. 2009;21:321-326.
12. Rebello J, Ohno M, Ukeda H, Kusunose H, Sawamura M. 3, 6 anhydrogalactose, sulfate and methoxyl contents of commercial agarophytes from different geographical origins. J Appl. Phycol. 1997;9:367-370.
13. Sousa AMM, Alves VD, Morais S, Delerue-Matos C, Gonçalves MP. Agar extraction from integrated multitrophic aquacultured *Gracilaria vermiculophylla*: evaluation of a microwave-assisted process using response surface methodology. Bioresource Technol.

2010;101:3258-3267.
14. Shukla MK, Kumar M, Prasad K, Reddy CRK, Jha B. Partial characterization of sulfo hydrolase from *Gracilaria dura* and evaluation of its potential application in improvement of the agar quality. Carbohydr. Polym. 2011;85:157-163.
15. Rao AV, Bekheet IA. Preparation of Agar-Agar from the Red Seaweed *Pterocladia capillacea* off the Coast of Alexandria, Egypt. Applied and Environmental Microbiology. 1976;32(4):479-482. https://doi.org/10.1128/aem.32.4.479-482.1976
16. Din SS, Chew KW, Chang YK, Show PL, Phang SM, Juan JC. Extraction of agar from *Eucheuma cottonii* and *Gelidium amansii* seaweeds with sonication pretreatment using autoclaving method. Journal of Oceanology and Limnology. 2019;37(3):871-880. https://doi.org/10.1007/s00343-019-8145-6
17. Usov AI, Yarotsky SV, Shashkov AS. I3C NMR spectroscopy of red algal galactans. Biopolymers. 1980;19:977-990.
18. Friedlander M, Lipkin Y, Yaphe W. Composition of agars from Gracilan'a cf. verrucosa and *Pterocladia capillacea*. Bat. Mar. 1981;24:595-598. Furneaux, R. H., I. J. Miller & T.
19. Wen ZC, Craigie JS. Composition and properties of agar-type polysaccharides from *Gracilaria sjoestedtii* Kylin. Chin. J Oceanol. Limnol. 1984;2:88-91.
20. Lahaye M. Agar from Gracilariu spp. PhD Thesis, McGill University, Montreal, Quebec, Canada, 1986, 330.
21. Gao HZ, Zepp R. Factors influencing photoreactions of dissolved organic matter in a coastal river of the southeastern United States. Environ. Sci. Technol. 1998;32:2940-2946.
22. Ursi S, Pedersen M, Plastino E, Snoeijs P. Intra specific variation of photosynthesis, respiration and photoprotective carotenoidsin *Gracilaria birdiae* (Gracilariales: Rhodophyta). Mar. Biol. 2003;142:997-1007.
23. Li H, Yu X, Jin Y, Zhang W, Liu Y. Development of an eco-friendly agar extraction technique from the red seaweed *Gracilaria lemaneiformis*. Bioresource Technology. 2008;99(8):3301-3305. DOI: 10.1016/j.biortech.2007.07.00
24. Araki CH. Acetylation of agar like substance of *Gelidium amansii*; J Chem. Soc. Japan, 1937, 1338-1350.
25. Araki C. Some recent studies on the polysaccharides of agarophytes. Proc. int. Seaweed Symp. 1966;5:3-17.
26. Rees DA, Welsh EJ. Secondary and tertiary structure of polysaccharides in solution and gels. Angew. Chem. Int. Ed. Eng. 1977;16:214-224.
27. Amott S, Fulmer A, Scott WE, Dea ICM, Moorhouse R, Rees DA. Agarose double helixand its function in agarose gel structure. J Mol. Biol. 1974;90:269-284.
28. Duckworth M, Yaphe W. The structure of agar. Part I. Fractionation of a complex mixture of polysaccharides. Carbohydr. Res. 1971b;16:189-197.
29. Duckworth M, Yaphe W. Preparation of agarose by fractionation from the spectrum of polysaccharides in agar. Anal. Chem. 1971a;44:636-641.
30. Young KS, Duckworth M, Yaphe W. The structure of agar. Part 111. Pyruvic acid, a common feature of agars from different agarophytes. Carbohydr. Res. 1971;16:446-448.
31. Lahaye M, Yaphe W. The chemical structure of agar from *Gracilaria compressa* (C. Agardh) Greville, *G. cervicornis* (Turner) J Agardh, *G. damaecornis* J Agardh and *G. domingensis* Sonder ex Kiitzing (Gigartinales, Rhodophyta). Bot. 1989 Mar;32:369-377.
32. Batey JF, Turvey JR. The galactan sulfate of thered alga *Polysiphonia lanosa*. Carbohydr. Res. 1975;43:133-143.
33. Djabourov M, Clark AH, Rowlands DW, Ross-Murphy SB. Small-angle X-ray scattering characterization of agarose sols and gels. Macromolecules. 1989;22:180-188.
34. Abdul Khalil HPS, Lai TK, Tye YY, Rizal S, Chong EWN, Yap SW, *et al*. A review of extractions of seaweed hydrocolloids: properties and applications. eXPRESS Polymer Letters. 2018;12(4):296-317. https://doi.org/10.3144/expresspolymlett.2018.27.
35. Surender Reddy K, Abraham A, Afewerki B, Tsegay B, Ghebremedhin H, Teklehaimanot B. Extraction of Agar and Alginate from Marine Seaweeds in Red Sea Region. International Journal of Marine Biology and Research. 2018;3(2):1-8. https://doi.org/10.15226/24754706/3/2/00126
36. Aini N, Munarso SJ, Annisa FS, Jayanthi TT. Analog Rice Characteristics of Corn Red Bean Flour Using Agar as a Binder. Agricultural Postharvest Research Journal. 2019;16(1):19.
37. Horiuchi H, Sugiyama J. Mechanical and Structural Properties of Agar-Gelatin Mixed Gels. Agricultural and Biological Chemistry. 1987;51(8):2171-2176. https://doi.org/10.1080/00021369.1987.10868363
38. Ramadhan W, Trilaksani W. Hydrocolloid Agar, Sucrose and Acidulant Formulations in The Development of Sheet Jam Products. JPHPI. 2017;20(1):95-108.
39. FDA. Agar-agar, GRAS (generally recognized as safe) Food Ingredients. Bethesda, MD, Food and Drug Administration, PB-221225 NTIS, US Department of Commerce, 1972.
40. FDA. Evaluation of Health Aspects of Agar-agar as a Food Ingredient. Bethesda, MD, Food and Drug Administration, PB-265502, Federation of American Societies for Experimental Biology, 1973a.
41. Kasfillah K, Sumarni W, Pratjojo W. Karakterisasi Edible Film Dari Tepung Biji Nangka Dan Agar-Agar Sebagai Pembungkus Jenang. Indonesian Journal of Chemical Science, 2013;2(3).
42. Sira KS. Agar-Agar (*Gelidium corneum*) as an Additive to an Emulsifying Agent in Photographic Silkscreen Printing. Psychology and Education. Published, 2021. http://psychologyandeducation.net/pae/index.php/pae/article/view/5152/4480
43. Roy S, Rhim JW. Agar-based antioxidant composite films incorporated with melanin nanoparticles. Food Hydrocolloids. 2019;94:391-398. DOI:10.1016/j.foodhyd.2019.03.03
44. Shankar S, Rhim JW. Preparation and characterization of agar/lignin/silver nanoparticles composite films with ultraviolet light barrier and antibacterial properties. Food Hydrocolloids. 2017;71:76-84. DOI:10.1016/j.foodhyd.2017.05.002
45. Shahruzzaman Mohammad, Sakib, Md Nurus, Biswas Shanta, Rahman Mohammed, Haque Papia, Mallik Abul. Pharmaceutical Applications of Agar-Agar, 2019. 10.1201/9780429328299-3.
46. Armisén R. Agar and agarose biotechnological

applications. International Workshop on Gelidium, 1991, 157-166. DOI:10.1007/978-94-011-3610-5_15
47. Chew KW, Juan JC, Phang SM, Ling TC, Show PL. An overview on the development of conventional and alternative extractive methods for the purifi cation of agarose from seaweed. Separation Science and Technology. 2018a;53(3):467-480, https://doi.org/10.1080/01496395.2017.1394881.
48. Ouyang QQ, Hu Z, Li SD, Quan WY, Wen LL, Yang ZM, *et al*. Thermal degradation of agar: mechanism and toxicity of products. Food Chemistry. 2018;264:277-283. https://doi.org/10.1016/j.foodchem.2018.04.098.
49. Armiseân R, Galatas F. `Production properties and uses of agar', in McHugh D. (ed.), Production and Utilization of Products from Commercial Seaweed. Rome, FAO Fisheries Technical Paper No. 288, 1987, 1±57.
50. Ghorbal Achraf, Grisotto Federico, Charlier Julienne, Goyer Cedric, Demaille Christophe, Palacin Serge, *et al*. Nano-Electrochemistry and Nano-Electrografting with an Original Combined AFM-SECM. Nanomaterials, 2013, 3. 10.3390/nano3020303.