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Agar-Agar Extraction, Structure, Properties And Applications: A Review

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Abstract

Agar is a phycocolloid component that is obtained commercially from the species Gelidium and Gracilaria. Agar was discovered in Japan in the mid-17th century, although its name is Malayan. In this review, we show the different properties of agar. Agar, a gelatinous polysaccharide in the cell walls of many red algal species, is widely used as a gelling, thickening, and stabilizing agent. Agars with the highest gel strength had the best performance. Agar-agar is a gel-forming polysaccharide that produces D-galactose when hydrolyzed with acid. Agar: Agar is a mixture of polysaccharides: 85% of agarose, a neutral polymer, and 15% of agaropectin, a charged sulfated polymer. Diverse applications are also presented, in the preparation of food for insects, for plant tissue culture, and in the preparation of culture media for microorganisms, as well as gels for denture molding, the reproduction of archaeological remains, or fingerprinting in police work. Seaweed materials with higher agar yields and better gelling properties are desired due to the growing demand for agar in the global market.

Keywords: Agar-Agar, Gelidium, Gracilaria, Gel Strength, ,Agarose, Agaropectin

1. Introduction

Japan is where agar first appeared in 1658. It was first brought to the Far East and then to the other nations that produced agarophyte seaweed. It was first utilized in Europe in 1859, and by 1882, it was being added to bacteriological culture. Because the production of hydrogen bridges, or physical gels, is the only mechanism behind the gelation and melting of agar and its fractions, including agarose and agaropectin, the process is remarkably reversible (Armisen & Gaiatas, 2009).

Since several centuries ago, agar, a phycocolloid that may be economically manufactured from Gelidium and Gracilaria species, has been used in industry to prepare solid microbiological media (Armisen, 1991).

In Japan's coastal regions, the use of several types of seaweed extracts that resemble agar gel most likely began in prehistoric times. The capacity of agar to make fruit and vegetable jelly was brought to Europe by Dutch immigrants to Indonesia. Robert Koch first presented agar as a culture medium to the globe in 1882 (Nussinovitch & Nussinovitch, 1997). Agar is extracted from some marine algae belonging to the class Rhodophyceae (Selby & Whistler, 1993). The phrase “agar-agar” originates from Malaya, and although it is also known as gelosa in French and Portuguese speaking nations, agar is the most often used term (Armisen & Galatas, 1987).

Agar was mostly imported from Japan by most nations towards the end of the 19th century, even though it is today produced in many other countries (Pandya *et al.*, 2022). Since Gracilaria species have high sulfate contents, they yield low quality agars. Because of this, many Gracilaria agar gel qualities need to be enhanced using the right procedures (Kim & Shin, 2017). The primary red-purple seaweed, Gelidiella acerosa, which is a member of the Rhodophyceae family, was initially discovered in 1964 by a survey team from M/s. Cellulose Products of India Ltd., Ahmedabad, in the Gulf of Mannar South, on the eastern sea coast of the Indian Peninsula. In the same area, Gracilaria Edulis, a secondary weed, is also widely distributed (Gopal, 1979).

In Taiwan, agar is often translated as "vegetable swiftlet" due to its similar texture to the swiftlet nest, which is used to make bird's nest soup (Lee *et al.*, 2017). Agar is another important natural polysaccharide that is produced from particular types of red algae and used as a component in food and microbial cultures (Betraoui *et al*., 2023). Discussion is held regarding developments in the chemistry and physico-chemical characteristics of agar since Arakis 1965 review at the Fifth international Seaweed Symposium (Lahaye & Rochas,1991). The Gelidiales (such as Pterocladia and Gelidium spp.) and the Gracilariales are the main suppliers of commercial agars (Lai & Lii,1997).

Agar, a polycationic biopolymer, is the most widely used medium for cultivating cells (Boral & Bohidar, 2009). The supply of fiber in China is not keeping up with the increasing demand for paper and paperboard goods. New sources of fiber were required due to the growing demand for paper products. In China , the red alga Gracilariopsis lemaneiformis is commercially grown on a huge scale for the purpose of extracting agar (Pei *et al.*, 2013).

2. Properties

agar agar, a hydrophilic colloid that is soluble in boiling water but insoluble in cold water (Selby & Whistler , 1993). The properties of bacterial-grade agar are influenced by the vast array of purifying methods available (Armisen, 1991). Agar- agar is regarded as an economical, non-toxic, biocompatible inert biopolymer (Sattar *et al.*, 2018). Agar is defined as a hydrocolloid that is soluble in boiling water with a clear aqueous solution at 1.5% (w/v) and forms a gel between 32 and 43 C that does not melt below 85 C by the US Pharmacopeia and the Food Chemicals Codex (Lee *et al.*, 2017). The hydrolysate of agar, known as agar oligosaccharides (AOs), exhibits a greater variety of bioactivities due to its decreased molecular weight, improved water solubility, and increased absorption efficiency. Compared to agar, agar oligosaccharides (AOs) have superior protein stabilizing activity and water solubility (Chen *et al.*, 2021). The methoxyl content of the sample mostly determines the agar gelation temperature (Boral & Bohidar, 2009).

2.1 Structure

Agar agar is a mixture of agarose and agaropectin, with agarose having the more desirable properties (Olatunji & Olatunji, 2020). From a structural perspective, agar- agar is a polymer of agarobiose made up of d-galactose and 3,6-anhydro-l-galactopyranose repeating units (Sattar *et al*., 2018). B-(1,4) and a-(1,3) links alternatively link L-galactoses,D-galactoses, and 3,6-anhydro-L-galactose,which make up the majority of the heterogeneous galactans in agar (Chi *et al., 2012).* Agar- derived oligosaccharides,also known as monosugars, generated by different agarases have emerged as a potentially interesting area of study because of their distinct biological properties, which include anti-fatigue, anti-cariogenic ,immunomodulatory, anti- tumor, antioxidant, and skin-whitening and skin-moisturizing properties (Park *et al*., 2020). Agaropectin and agarose both have a galactose- based backbone, agaropectin has been extensively modified with acidic side-groups like pyruvate and sulfate, whereas agarose has a neutral charge (Raphael *et al.*, 2010). Araki and his group have researched the main structure. Agarophyte red algae can be treated with alkali to extract agarose, a linear polymer (Nishinari & Fang, 2017). This agar agar polysaccharides molecular makeup is widely understood (Boral & Bohidar, 2009).

2.2 Extraction

Agar was utilized far less in laboratories than it was in food (Pandya *et al.*, 2022). Because it contains pigments, agar extracted with the conventional alkaline pretreatment always has a yellowish appearance (An *et al.*, 2021). There are five steps involved in removing agar from seaweeds: (1) Agar is washed, dried, and chemically treated; (2) Agar is heated to extract water; (3) Seaweed residues are filtered out; (4) Agar gel is cooled, frozen, and thawed; and (5) The solid agar is washed, bleached, and dried (Lee *et al*., 2017).

2.2.1. Alkali Treatment of Seaweeds

According to the socioeconomic background of agar development, the widespread usage of Gracilaria verrucosa, collected in Tokyo Bay during the postwar period, contributed to the development of agar hydrogel techniques worldwide. The first people to independently develop the alkali treatment method for the agar industry and apply it to the extraction of carrageenan were Kojima and Funaki at the Tokyo Institute of Technology. Alkali pretreatment is utilized to enhance the gelling ability of the agar family, even though it reduces yield and produces an effluent that could pose a risk to the environment if left untreated. With the aid of an alkali treatment, the mucilaginous substance resembling agaroid in Gracilaria is converted to agar at 85–90 °C through the suggested exchange process and partial elimination of sulfates. R and R" are polysaccharide radicals found in Gracilaria. It is believed that Gelidium species possess a high concentration of (l)-form polysaccharides that have the ability to gel significantly, while Gracilaria species are assumed to include a high concentration of (H)-form polysaccharides that do not. Alkali treatment of Gracilaria plants can provide a substitute product (l). Consequently, an aqueous solution of sodium hydroxide containing a small amount of ionized calcium is used. Better grade agar is produced by pretreatment with alkali; even after open boiling, it leaves very little insoluble debris and is readily soluble in water. It was proposed that the agar extraction method alkaline pretreatment help change the molecular weight by transforming L-galactose sulfate into 3,6-anhydrogalactose (Pandya *et al*., 2022).

2.2.2. Microwave-assisted extraction (MAE)

The microwave-assisted extraction (MAE) of agar from Gracilaria vermiculophylla, which was produced in an integrated multitrophic aquaculture (IMTA) system for Ria de Aveiro (northwest Portugal),. Using a 24 orthogonal composite design, the effects of the MAE operational parameters (temperature, stirring speed, solvent volume, and extraction time) on the agar's physical and chemical properties (yield, gel strength, gelling and melting temperatures, as well as sulfate and 3,6-anhydro-l-galactose contents) were assessed. The extracted agar quality was significantly lower than that of the conventional extraction method (2 hours at 85 °C), and it required less waste to be disposed of and less time to extract (Sousa *et al.*, 2010).

The modified method involved rinsing sun-bleached seaweed in water, grinding it to a pulp, and then soaking it for 24 hours before rinsing it again. After adding acetic acid to raise the pH to 6, the pulp was then extracted using water (weed-to-water ratio: 1:30) under pressure for two hours. Following freeze-thawing, the agar gel was bleached with NaCLO and then dried in a hot air current. It was discovered that pretreating the seaweed with alkali at 80 °C for two hours before extraction greatly enhanced the agar quality (Rao & Bekheet, 1976). Leaching the dried Gracilaria in boiling water, filtering the extract, and separating the agar by freezing and thawing to remove the water make up the standard protocol for agar extraction (Kumar & Fotedar, 2009).

Using terrestrial and marine biomass, new eco-friendly extraction techniques like ultrasound- assisted extraction, microwave-assisted extraction, surfactant-induced coagulation, or enzyme-assisted extraction have recently been investigated. These techniques may have financial, operational, and environmental benefits , but they must be chosen based on the target compound to be extracted as well as the characteristics of the raw materials (Martinez-Sanz *et al.*, 2021).

3. Application

Avoiding interactions with other components of the media, such as proteins, amino acids, sugars, other carbohydrates, meat extract, peptones, pigments, indicators, inhibitors, mineral salts, etc., is crucial because agar is only used as a gelling agent in solid media (Armisen & Galatas, 1987). After Arakis seminal study on the chemical structure of agar revealed that agarose is the primary component responsible for gelation, agar is being used as a gelling agent in more scientific and research fields (Pandya *et al*., 2022). Because of its rheological properties, agar agar is used in the food, biotechnology, and pharmaceutical industries as a thermoreversible gelling agent, stabilizer, texture modifier, and thickener (Olatunji & Olatunji, 2020).

Agar-agar, or Agr, is a gelling agent that increases crop output and functions as an amazing soil water-maintaining substance (Chaudhary *et al.*, 2020). Because of its strong gelling power and lack of reactivity with other biomolecules,agar agar is favored for commercial use in the food, pharmaceutical, and biotechnological industries (Sattar *et al.*, 2018).

Agar agar is a biological polymer that is commonly used in tissue engineering and pharmaceutical research for possible use in bone replacement. It is a hydrophilic natural polysaccharide that is easily fabricated by thermal crosslinking to hydrogel, which can also be used as a drug delivery system. Agar can also be used as a base for non-melting and non-disintegrating assumptions (Senthilarasan *et al*., 2014). Due to its jellifying qualities, it is a significant part of red algaecell walls and has been applied in numerous industrial and scientific settings (Chi *et al.*, 2012).

Agar:Agar is largely used in pharmaceuticals as a gelation, stabilization, and thickening agent. Furthermore, Anon agar is frequently used as a surgical aid and for purgative purposes. Researchers have worked hard to produce agar-based products for application in the pharmaceutical industry, including nanocomposite films and composite hydrogels. Agar was used to create an injectable, phase-changing composite hydrogel that might be used for photothermal and chemotherapy treatments of malignancies (Shahruzzaman *et al*., 2019).

Size-exclusion Chromatography, gel-bead filtration, and electrophoresis are used to separate proteins, lipoproteins, enzymes, and other high-molecular-weight molecules from one another using agar, especially agaran (Selby & Whistler, 1993). Agar has been applied in many different sectors and is a significant polysaccharide biomass. An essential step in the utilization of marine biomass and the preservation of the biosphere's carbon cycle is the enzymatic breakdown of agar. Significant advancements have been made in the biochemical analysis of agaroses and the isolation of agarolytic microbes as a result of several attempts (Chi *et al.*, 2012).

Agar is used in the culinary, cosmetic, biopharmaceutical, and regenerative industries all over the world because of its strong gelling ability, high hysteresis, and gel reversibility. When used as a food ingredient, yellowish agar lessens the visual appeal of food to customers. When used as a microbiological culture medium, it makes colony observation and counting more difficult (An *et al.*, 2021). Agar's primary applications include the creation of thermoreversible gels at low water concentrations. Additionally, it demonstrates a wide range of advantageous biological properties, such as immune-modulating, antiviral, antioxidant, anticoagulant, and anticancer properties (Capillo *et al.*, 2017).

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