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Effect of Extraction Methods on the Characteristics of Agar from *Gelidiella acerosa* (Forssk.) Feldm harvested from Gulf of Mannar in India.

Efecto de los métodos de extracción sobre las características del agar de *Gelidiella acerosa* (Forssk.) Feldm cosechado en el golfo de Mannar en la India.

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ABSTRACT

Agar is a sulfated polysaccharide extracted from red macroalgae of the species Rhodophyta. These polysaccharides are extensively used in food and pharmaceutical industrial applications. Further application potentials of this hydrocolloidal polymer in packaging industry due to its nontoxic and ecofriendly characteristics open up new opportunities to explore the benefits of these versatile products in uprising arenas. Comparison and systematic impact analysis of different extraction methods and their effects on basic properties of the extracted polysaccharides needs to be studied. *Gelidiella acerosa* (Forssk.) Feldm samples were collected from Mandapam and Pamban regions in the Gulf of Mannar on the southwestern coast of India. Cleaned, dried and powdered samples were used for polysaccharide extraction. Extraction yield, moisture, optical characteristics, solubility and structural characterization by FTIR-ATR were carried out for agar obtained by different extraction methods AE1, AE2 and AE3 respectively. The variation in the characteristics and properties of polysaccharides obtained by standard methods of extraction identifies AE2 as the best method based on extraction yield and spectral properties. This study paves the way for quality polysaccharides to be extracted for bulk processing considering the final application properties in existing and uprising sectors.

Keywords: *Gelidiella acerosa*, Sulfated Polysaccharide, Hydrocolloid, Characterization, Agar, FTIR-ATR.

RESUMEN

El agar es un polisacárido sulfatado extraído de macroalgas rojas de la especie Rhodophyta. Estos polisacáridos se utilizan ampliamente en aplicaciones industriales farmacéuticas y de alimentos. Los potenciales de aplicación adicionales de este polímero hidrocoloidal en la industria del embalaje debido a sus características no tóxicas y respetuosas con el medio ambiente abren nuevas oportunidades para explorar los beneficios de estos productos versátiles en arenas emergentes. Es necesario estudiar la comparación y el análisis sistemático del impacto de los diferentes métodos de extracción y sus efectos sobre las propiedades básicas de los polisacáridos extraídos.

Se recolectaron muestras de *Gelidiella acerosa* (Forssk.) Feldm de las regiones de Mandapam y Pamban en el Golfo de Mannar en la costa suroeste de la India. Se utilizaron muestras limpias, secas y pulverizadas para la extracción de polisacáridos. El rendimiento de extracción, la humedad, las características ópticas, la solubilidad y la caracterización estructural por FTIR-ATR se realizaron para agar obtenido por diferentes métodos de extracción AE1, AE2 y AE3 respectivamente. La variación en las características y propiedades de los polisacáridos obtenidos por métodos estándar de extracción identifica a AE2 como el mejor método basado en el rendimiento de extracción y las propiedades espectrales. Este estudio allana el camino para la extracción de polisacáridos de calidad para el procesamiento a granel considerando las propiedades de aplicación final en sectores existentes y emergentes.
Palabras clave: Gelidiella acerosa, Polisacárido Sulfatado, Hidrocoloide, Caracterización, Agar, FTIR-ATR.

INTRODUCTION

Agar is one of the commercially valuable hydrocolloidal polysaccharide extracted from the cell walls of red macroalgae of genera Rhodophyta such as *Gelidium* and *Gracilaria* spp. Agar is a polymeric compound that constitutes agarose and agaropectin (Kraan 2012, Yarnpakdee et al. 2015; Ganesan et al. 2017; Öğretmen et al. 2019). D and L-galactose are the major elements of this polysaccharide, with sulphate ester groups at every tenth D-galactopyranose structure. (FAO/JECFA 2006). Naidu (2000) explicates agar as a mixture of linear polymer, agarose composed of 3,6 anhydro-*α*-*L*-galactopyranose units linked at 1 and 4, β-D-galactopyranose units linked at 3 and 6 with the non-gelling agaropectin fraction contains sulfate ester, *D*-galactose with 3,6 anhydro-*L*-galactose. Joint FAO/WHO Expert Committee on food additives monogram (2006) describes agar as a hydrocolloid soluble in boiling water but insoluble in cold water.

Though commercial mariculture of several agarophyte species has been successfully established in India, *Gelidiella acerosa* is still harvested from wild biomass all over the world, despite rising raw material demand (Ganesan et al. 2017). Agar from *Gelidium* spp is utilized mostly for bacteriological and pharmaceutical industry because of its high-quality gel forming properties wherein, agar from *Gracilaria* is mostly utilized in food industries (Rodríguez et al. 2009, Heydari et al. 2014, Ganesan et al. 2017, Öğretmen et al. 2019).

Agar is endowed with versatile application potentials (Öğretmen et al. 2019). It is mainly utilized as stabilizer and gel forming substance in food, cosmetics, pharmaceutical products as well as in biotechnology and medical research (Chew et al. 2018).

The main objective of this paper is to assess the effect of commonly used extraction methods on the basic properties including moisture holding capacity, optical properties, solubility as well as to investigate the precise changes in the functional group of the resulting sulphated polysaccharide.

MATERIALS AND METHODS

Gelidiella acerosa (Forssk.) Feldm samples were collected in December at Mandapam and Pamban regions, Gulf of Mannar in the southwestern coast of India.

Gelidiella acerosa samples procured from the field were primarily given a wash with seawater and sun dried for 12 hours. The samples were then transported to the study location and washed thoroughly to remove the sand, dirt, debris and epiphytes. The samples were then dried at room temperature for 24 hours and at 45°C in cabinet drier until they reached a constant weight.

Extraction of Agar from *Gelidiella acerosa* (Forssk) Feldm: Extraction of Agar involves dissolving the colloidal substances from the cell wall of the algae into hot water under pressure followed by separation through freeze-thawing.

Agar Extraction Method – 1(AE1): Agar from the dried samples of *Gelidiella acerosa* (Forssk) Feldm was extracted with the modified procedure suggested by Prasad et al. (2006). Initially, 10 g of roughly powdered samples in triplicates were soaked in 500 ml of distilled water for 2 hours followed by pretreatment with 0.5 % acetic acid at 16-20°C for an hour. The contents were heated under pressure at 120°C for 1.5 hours. The filtrate was allowed to gel at room temperature and kept at -20°C for 15 hours. The frozen gel was thawed and the liquid was squeezed out, then washed with distilled water and air dried for 24 hours at room temperature before being dried in a cabinet drier at 60°C until constant weight.

Agar Extraction Method – 2(AE2): The modified method of Roleda et al. (1997) was used to extract agar from *Gelidiella acerosa* (Forssk) Feldm. 10 g of roughly powdered dried samples in triplicates were soaked in 300 ml of 1N Sodium Hydroxide for an hour at 90°C followed by neutralization with 300 ml of 0.5 % acetic acid at 16-20°C for another hour. The samples were rinsed thoroughly with tap water after each pretreatment and neutralization. Extraction with 300 ml of boiling water under pressure of 15-20 psi was carried out for one hour respectively. The contents were filtered, cooled and frozen overnight. The frozen gels were thawed, liquid was squeezed off and were air dried for 24 hours at room temperature at 30°C followed by drying in the oven at 60°C until the weight remained constant.

Agar Extraction Method – 3(AE3): For the extraction of agar from *Gelidiella acerosa* (Forssk) Feldm the procedure used by Ganesan et al. (2008) was adopted with minor modifications. 10 g of roughly ground power of seaweed samples in triplicates were added to 200 ml of distilled water and boiled for 2.5 hours under pressure of 15-20 psi. The extracts were then filtered and allowed to cool at room temperature. The gels were frozen overnight in a refrigerator and thawed for 24 hours. The thawed contents were drained and dried at room temperature for air dried for 24 hours at room temperature 30°C followed by drying in the oven at 60°C until constant weight was achieved.

Basic Characteristics of Extracted Polysaccharides

Extraction Yield: The total extraction yield percentage of agar was determined from the final weight of the polysaccharide obtained by the initial weight of the dried sample with a multiple of 100. The triplicate extraction yield percentage values were used to calculate the mean with standard deviation.

$$\text{Yield of Polysaccharide } (\%) = \frac{\text{Final weight of the polysaccharide}}{\text{Initial weight of the dried seaweed}} * 100$$

Moisture: The moisture content of the extracted agar was estimated through methods AE1, AE2 and AE3 from *G. acerosa* with Shimadzu Electronic Moisture Analyzer (MOC-120H).

Optical Properties: Optical properties of the extracted agar using different extraction techniques were evaluated using Precise Color Reader which measures L*, a* and b* values. Lightness is expressed in terms of L* values as white (100) to black (0). Wherein, a* values range from -60 (green) to +60 (red) also b* values from -60 (blue) to +60 (yellow) (CIELab 1976). The instrument was validated using standard black and standard white calibration plates prior to each set of measurements (Wrolstad and Smith 2017, Tim 2018). The optical properties of each sample were assessed for at least three points on the sample surface.

Solubility: The ability of the extracted agar from AE1, AE2 and AE3 to form homogenous solution in the solvent medium was assessed with known volumes of polar and non-polar solvents.

Characterization with FTIR -ATR Spectroscopy: The functional group present in the extracted agar samples were interpreted from Shimadzu Fourier Transform Infrared Spectroscopy in the mid infrared wavelength range from 400 to 4000 cm^{-1} with 1 cm^{-1} data interval levels using Shimadzu Miracle10 Fourier Transform Infrared Spectroscopy accompanied with Attenuated Total Reflectance (ATR) system with the incident angle of 45°. A pinch of the sample was positioned on the zinc selenide coated ATR surface sample holder. Each spectrum is the average of 45 scans, ratioed and taken against the blank background with the empty ATR system performed prior to each set of samples to eliminate environmental interference (Volery et al. 2004). The results will be given as percentage transmittance with the resolution of 16 cm^{-1} for each sample.

RESULTS AND DISCUSSION

Extraction Yield: The table 1 compares the extraction yields, moisture content and optical properties of agar produced from different extraction methods. The average extraction yield percent of agar prepared from *G.acerosa* using various extraction method such as AE1, AE2 and AE3 were 19.1±0.4, 18.2±0.4 and 9.6±0.2 respectively and were statistically significant at less than 1 percent level ($P<0.001$). Prasad et al. (2006) found comparable results for the extraction of agar from *G.acerosa* harvested in December, with yields ranging from 8.5 to 31.5 % in different locations around the Gujarat coast in India. The AE3 reported least yield of 9.6 % when compared to the other two methods which could be due to the absence of the pretreatment process which is par with of study of Roleda et al. (1997) done in Philippine. The extraction yield of the present study is comparatively higher and relative to the study of Prasad et al. (2007) with pretreatment.

Moisture: The average moisture content of agar obtained from *G.acarosa* after complete drying to constant weight at 65°C in triplicate analysis were 14.1% (AE1), 14.2 % (AE2), and 14.3 % (AE3) with no significant difference. The present extraction methods had the least impact on moisture holding capacity, as seen by minute changes in moisture content.

Optical Properties: The color of agar observed in color reader from different extraction methods were statistically significant at less than 1 percent level and are depicted in table 1. The lightness (L*) values which indicate

the black to white color observed at the range from 22.75 to 37.87. Similarly, the optical property values (a*) from 0.46 to 1.45 indicating a mild red color and with the optical property values (b*) reported from 8.64 to 12.20 in various extraction methods corresponding to dull pale-yellow color represents the final color of pale yellowish color red hue. The color of agar obtained from extraction methods AE1, AE2 and AE3 are in par with the given specification given by FAO/JECFA (2006). According to the given specification, agar could be light yellowish orange, yellowish grey to pale yellow in color or it can be colorless and the color of the powdered agar could be pale or yellowish white to white in color.

Table 1: Characteristics of Agar obtained by different Extraction Methods

Extraction Method	Yield (%)	Moisture (%)	Optical Properties		
			L*	a*	b*
AE1	19.11±0.45***	14.10±0.20ns	28.33±0.21***	0.46±0.19***	8.64±0.20***
AE2	18.24±1.4***	14.15±0.05 ns	22.75±0.03***	1.45±0.40***	10.56±0.36***
AE3	9.69±0.27***	14.30±0.04 ns	37.76±0.63***	0.48±0.08***	12.21±0.25***

L*-Lightness (0 .black to 100 .White); a* (-60 .green to ±60 .red); b* (-60 .blue to ±60 .yellow)

*** Indicates 'P' values significant at <0.001% Level ns-Not Significant

Table 2. Wavenumber (cm^{-1}) and Bonding Nature of FTIR-ATR spectra of Extracted Agar

Wavenumber (cm^{-1})	Extraction Method	Intensity	Bonding Nature
3371	AE2	Strong	O-H Stretch~
3363	AE3	Strong	O-H Stretch~
3348	AE1	Strong	O-H Stretch~
2129	AE1, AE2, AE3	Weak	C=C Stretch~
2113	AE2, AE3	Weak	C=C Stretch~
1643	AE1, AE2, AE3	Strong	C=C Stretch~
1303	AE1	Weak	C-O-C Stretch †, S=O Stretch~
1064	AE2	Medium	C-O-C Stretch†, S=O Stretch~
1056	AE3	Medium	S=O Stretch~, C-C/C-O/C-O-S Stretch+
686	AE1, AE2, AE3	Strong	S-O Stretch, C-S link vibration+
455	AE2	Medium	S-S Stretch*
439	AE1, AE3	Medium	S-S Stretch*

*Coates, J. ,2006 ~ www.sigmaaldrich.com †Hussein., 2015 +Shahnaz et al.2019

Solubility: Agar extracted through AE1, AE2 and AE3 methods exerted similar properties. They were completely dissolved in hot boiling water, while they just absorbed water but did not dissolve in cold water. This

statement is similar to the recommendation of FAO/JECFA (2006) that the extracted agars are insoluble in known non-polar solvents such as ethanol and chloroform.

Characterization with FTIR-ATR Spectroscopy: The Attenuated Total Reflectance aided infra-red spectra records for the agar samples extracted with methods AE1, AE2 and AE3 are given in the figure-1 and table 2.

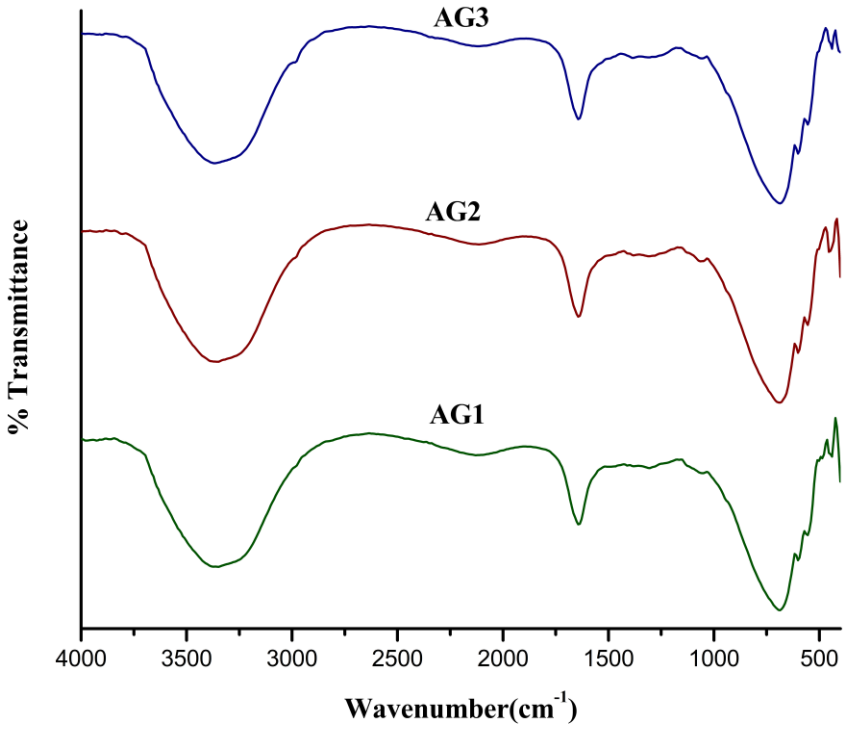

Figure 1. FTIR-ATR Spectra of Agar-AE1, AE2, AE3.

A relatively strong band stretching noted in the region around 3350 cm^{-1} especially at 3371 cm^{-1} for AE2 ,3363 cm^{-1} for AE3 and 3348 cm^{-1} for AE1 indicates the presence of O-H. The existence of prominent peaks at 1643 cm^{-1} indicates the presence of C= O and NH groups that signify conjugated peptide bonds (Meena et al. 2011). The peak noted around 1064 cm^{-1} and 1056 cm^{-1} also denotes C-O-C vibration by 3,6 anhydro galactopyranose bridge and due to the residues of D-galactose (Hussein 2015). Also, the standard peaks noted by Meena et al. (2011) in sigma agarose sample represents similar pattern of strong and prominent peak arrangements at 3434 cm^{-1}, 1642 cm^{-1}, 1075 cm^{-1} and medium to weak peaks at 2144 cm^{-1}, 1309 cm^{-1}, 658 cm^{-1},539 cm^{-1} and 458 cm^{-1} were noted . The medium band spectra noted at 1056 cm^{-1} for AE3 and 1064 cm^{-1} in AE2 denotes the C-O ether bond distinctive to 3,6 anhydro galactose stretching vibrations which is also noted by Shahnaz et al. (2019) in agar. Whereas, the peaks noted in the agar obtained through AE1, AE2 and AE3 are given in table-2 are notably same with precise peaks at 3350 cm^{-1}, 1643 cm^{-1} and 686 cm^{-1} indicating the production of good quality of agar in all three methods.

Agar extracted from *Gelidiella acerosa* (Forssk.) Feldm with method AE1 revealed maximum extraction yield of 19.1±0.4 %. No significant changes were seen in moisture content and solubility of agar extracted from AE1, AE2 and AE3. The color of the extracted agar was within the standard range in all three trials. Significant difference at less than 1 percent level was noted among the agar extracted through all three methods in terms of yield and optical property. Specific changes were noted through the wavenumber and transmittance band obtained from infra-red spectra recorded for AE1, AE2 and AE3 respectively. The medium band spectra noted at 1056 cm^{-1} for AE3 and 1064 cm^{-1} in AE2 denotes the C-O ether bond distinctive to 3,6 anhydro galactose stretching vibrations. This observation denotes the quality of agar obtained using methods AE2 and AE3. But, relatively small mass of agar obtained from method AE3 brings it to the dismissive side. In turn, agar extracted through AE2 makes it acceptable in terms of yield and quality also can be utilized to explore new opportunities and obscure application strategies in imminent future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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