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Effects of Season on the Yield and Properties of Agar from *GRACILARIA CORTICATA*

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***Abstract:*-- Agars are well-known as water-soluble, gel-forming polysaccharide extracts from agarophyte members of red seaweeds. Gracilaria corticata is one of the naturally occurring agarophytes of Indian waters. Seasonal changes in the yield and properties of agar were studied from Gracilaria corticata growing at Tuticorin coast, Gulf of Mannar. Plants were collected during the seasons of premonsoon, monsoon and postmonsoon. Agar yield, gel strength, gelling and melting temperatures, sulphate and 3,6 anhydro-galactose content were determined. Carbohydrate content was measured from algal tissue. There was a clear seasonality in the yield and properties of agar. Agar yield of Gracilaria corticata ranged between 6.9% and 33.5%. Agar yield increased from premonsoon to monsoon season and lowest yield was found in the postmonsoon season. Gel strength ranged between 42 and 69 g cm-2. Gel strength decreased from premonsoon to monsoon and was highest during the postmonsoon season. An inverse relationship was found between agar yield and gel strength. The gelling and melting temperature ranged from 35oC to 38oC and 56.5oC to 70.5oC respectively. Maximum content of 3,6 anhydrogalactose and sulphate were observed in monsoon season. The result indicates clear seasonal variations in yield and properties of agar from Gracilaria corticata.**

***Keyword:*-- Agar yield, Gel strength, Melting and gelling temperatures, 3,6-anhydrogalactose.**

1. INTRODUCTION

Marine macroalgae, generally referred to seaweeds, classified by their pigmentation, morphology, anatomy, and nutritional composition as red (Rhodophyta), brown (Phaeophyta) or green seaweeds (Chlorophyta) [1]. About 250 macroalgal species have been commercially utilized worldwide and about 150 species are consumed as human food [2].
One of the oldest groups of eukaryotic algae is the Rhodophyta (red algae). Some Rhodophyta known as agarophyte produce phycocolloid agar in their cell walls. The taxonomic classification of agarophytes in the class of Florideophyceae is divided into three orders, Gelidiaceae (Gelidium, Gelidiella, Pterocladia), Gracilariales (Glacilaria) and Ahnfeltiacea (Ahnfeltia) [3].

The order Gracilariales (Gracilaria) is the chief universal agar source for agar extraction. McHugh (1991) reported that the quantity of agar produced from Gracilaria and Gelidium was the largest in the world which was 53% and 44%, respectively, compared to other agarophytes like Gelidiella and Pterocladia which only produced a small quantity (3%) of agar [4]. The best quality agar is extracted from the genus Gelidium, but because of the high cost and insufficient wild stock, Gracilaria has now been subjected to numerous structural studies of its agar due to the excellent substitute for Gelidium agar in the food industry.

The important and commonly occurring agarophytes of India are Gelidiella acerosa, Gracilaria edulis, G. crassa, G. verrucosa, G. corticata and G. folifera. Of these, only Gracilaria edulis and Gelidiella acerosa are used now as raw material for the production of agar in India, since they are rich in agar content with good gel strength and also available in exploitable quantities [5].

Easy availability of wild Gracilaria spp has led to the principal source of agar worldwide [3]. Generally, Gracilaria spp. yields low quality agar due to high sulphate content and therefore they are called 'agaroids' or 'Gracilaria gum' [6]. It is well known that quantity and quality of phycocolloid varies not only among species [7], but also due to influence of environmental factors [8], seasonal variations [9] and extraction methods [10].

Quality of agar is the sole criteria for its price, which is decided by gel strength, sulphate content and melting point of agar. Hence, any value addition to the indigenously produced agar such as sulphate content reduction and gel strength increase, not at the expense of yield will definitely make agar industry economically viable.

Agar polysaccharides isolated from Gracilaria are typically more sulphated than those obtained from Gelidium and Pterocladia. During the biosynthesis of agar polymers the L-galactose 6-sulphate content of the native agars varies seasonally and alters its gelling behavior [11]. In recent years Gracilaria species have been employed successfully for the production of food grade agar and this success has been due to the use of alkaline treatment to improve the agar quality [12]. The present study was under taken to determine

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seasonal variations in the yield, gelling characteristics and chemical composition of agars from Gracilaria corticata.

MATERIALS AND METHODS

Gracilaria corticata (J. Agardh) was collected during premonsoon period (July, August and September), monsoon period (October, November and December) and postmonsoon (January, February and March) period from Red gate to light house stretch of Tuticorin coast. The collection was made during the morning low tide. They were transported to the laboratory in plastic bags, thoroughly washed with freshwater and spread for shade drying. The shade dried materials were powdered and stored in polythene covers for analysis. Agar was extracted from the powdered material and its properties were analyzed using standard procedures.

Extraction of Agar [13]

10g of bleached sun dried seaweed sample was cut into small pieces, washed twice and put in a 2 litre conical flask. 300ml of distilled water was added and covered with a cotton plug. The conical flask was kept in an autoclave and cooked for 1-2 hours at 20lb pressure. The extract was filtered in trays using organdy cloth and cooled at room temperature. The gel was then cut into strips and frozen in a refrigerator for one day. The frozen gel was thawed and dried in the sun using nylon screen frames.

The agar yield was calculated by using the following formula.

% yield = Total weight of agar/weight of the seaweed powder×100

Physical properties [10]

Dry agar was ground in a Tecator mill and reconstituted in 1.5% w/v solutions to measure the physical properties (gel strength, melting and gelling temperature)

Gel strength

Gel strength was measured after gelling overnight at room temperature by measuring the load (g cm-2) causing a cylindrical plunger (1 cm cross section) to break a standard gel in 20 s.

Gelling temperature

Gelling temperature was obtained by the addition of 10 mL hot agar solution into a test tube (2.3 cm diameter, 6 cm height). A glass bead (5 mm diameter) was placed in the test tube. The tube was tilted up and down in a water bath at room temperature until the glass bead ceased moving. The gel temperature in the tube was immediately measured by introducing a precision thermometer (0.1 oC divisions).

Melting temperature

Melting temperature of the gel in a test tube (2.3 cm diameter, 16.5 cm height) was measured by placing an iron bead (9 mm diameter) on the gel surface. The test tube was clamped in a water-bath and the temperature raised from 50 to 100 0 C. The melting point was recorded with a precision thermometer when the bead sank into the solution.

Estimation of Carbohydrate [14]

Procedure

100mg of the dry seaweed powder was ground with 5ml of 80% methanol. The extract was centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and made up to 10ml with 80% of methanol. To this 10ml of petroleum ether was added and mixed well. The lower layer was taken for carbohydrate estimation.

To 0.1 ml of the extract, 4.9 ml of anthrone reagent (0.2% w/v in conc H2So4) freshly prepared was added and kept in a boiling water bath for 10 minutes. After cooling, the O.D was read at 625nm using Spectrophotometer (UV –VIS Spectrophotometer SL 150). Glucose was used as the standard.

Chemical properties

Estimation of 3,6 Anhydrogalactose [15]

Reagents

Acetol (or) 1,1-Diethoxyethane

Stock A

100µ litre of acetol was made up to 10.3ml with glass distilled water.

Stock B

From the stock (A), 0.1ml was made up to 2.5ml with glass distilled water.

Resorcinol

150mg of resorcinol was dissolved in 100ml distilled water and kept in refrigerator.

Acetol-Resorcinol

This reagent was prepared by mixing 100ml of concentrated Hcl, 9.0ml resorcinol and 1.0ml of acetol stock (B). This mixture was always prepared fresh.

Procedure

To 100mg of the sample taken in an ice bath, 5.0ml of cold acetal resorcinol reagent was added and mixed thoroughly. Then the sample was kept in an oven maintained at 80oC for 30 minutes for the development of permanent light violet colour and transferred back to the ice bath. After 3 minutes the OD was read at 555nm in a spectrophotometer. The amount of 3,6 Anhydrogalactose was determined using a standard graph prepared with different concentration of D-fructose (10-100 µg/ml).

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Estimation of sulphate [16]
Turbidimetric method
Reagents

a) 6M Hcl
b) 1N Hcl
c) 70% Sorbitol
d) Barium chloride

Procedure

10g of sample was homogenized with 6ml of 1N Hcl and transferred to a hydrolyzing tube sealed properly and subjected to hydrolysis in a boiling water bath for a period of 4 hours. The sample was then cooled to room temperature. The solution was filtered through whatman No-1 filter paper made up to 25ml with glass distilled water.

To 1.0ml sample, 0.1ml of 6M Hcl and 0.5ml 70% sorbitol was then mixed with a magnetic stirrer, during stirring 1.0g Barium chloride crystals was added and then read at 470nm within 1 minute. The amount of sulphate in sample was calculated from a standard graph prepared with potassium sulphate ranging from 10-100µg/ml.

Statistical Analysis

All the biochemical parameters were estimated on triplicate determinations. Standard deviation and standard error were calculated following Zar, [17].

(i)Standard deviation SD = $\sqrt{\frac{\sum d^2}{N-1}}$

Where d refers to the deviation from mean and N the total number of observations.

(ii)Standard error SE = $\frac{SD}{\sqrt{n-1}}$

Where SD refers to the standard deviation and n the total number of observations.

RESULTS AND DISCUSSION

Variations in the yields, gelstrength and gelation characteristics of the agars, have been demonstrated to be dependent on time of season and life stages of the alga [18]. The results obtained on the seasonal variations in the yield and properties of agar from Gracilaria corticata are presented in Fig. 1-7.

Agar yield of Gracilaria corticata ranged between 6.9% and 33.5%. Maximum agar yield of 33.5% was obtained in the monsoon period followed by the premonsoon period (24%). This finding is quite similar to earlier studies. Villanueva et al [19] reported that the yield of agar from Gracilaria eucheumoides was at a maximum during rainy season. Freile-Pelegrín and Robledo [9] found maximum agar yield from Gracilaria cornea during rainy season. Most remarkable seasonal effect in agar yield was observed in Gracilaria corticata during the rainy season. A relationship possibly exists between the yield and rainfall, since there is a reduction in salinity in the rainy season. Bird [20] has proposed that in low salinity the agar deposition between cell walls might provide additional structural support for turgid cells. Such an increase in agar yield under reduced salinity has also been observed by Luhan [21]. Chennubhotla et al [22] reported maximum agar yield from Gracilaria edulis in winter and it was minimum in summer. Pondevida and Hurtado-Ponce [23] reported that agar yield varied with seasons and higher agar yield was obtained during winter season.

The minimum agar yield of 6.9% was obtained in the post monsoon period in the present study. In general, agar yield declined during high seawater temperature in summer or during periods of rapid growth [24].

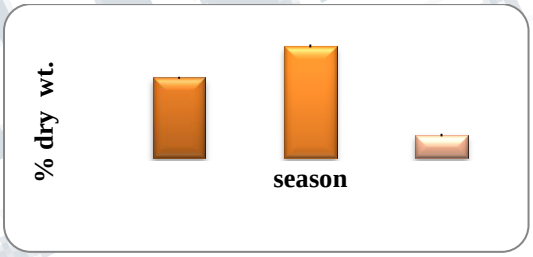

Fig. 1. Seasonal variation in agar yield of Gracilaria corticata

Maximum carbohydrate concentration was recorded from Gracilaria corticata collected from monsoon period (53.6%) followed by premonsoon period (50.3%) and post monsoon period (49.5%). Freile-Pelegrín and Robledo [9] found highest carbohydrate content during the rainy season and coincided with the maximum agar yied. A similar relationship was found for Gracilaria tikvahiae [25]. This close relationship between total carbohydrate and agar may provide an accurate indicator of agar yield in the alga.

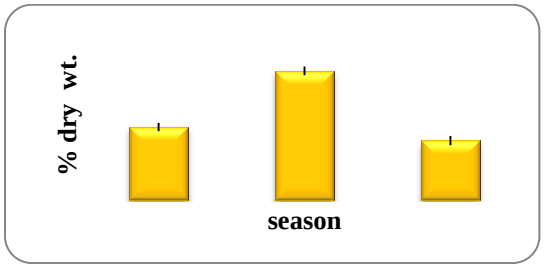

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Fig. 2. Seasonal variation in carbohydrate content of Gracilariacorticata

A seasonal fluctuation was also observed for gel strength. This observation is in agreement with reports on other agarophytic species [26, 27]. The gel strength of Gracilaria corticata was found to be between 42 and 69g/cm2. The higher gel strength (69 g/ cm2) was observed in the post monsoon season and lower gel strength (42 g/cm2) was observed in the monsoon season followed by premonsoon season (54 g/ cm2). Nelson et al [28] reported that Gracilaria lichenoides from Saipan produced a high-quality agar at relatively high seawater temperature. In contrast, Karthikeyan and Eswaran [29] reported that, gel strength was higher in monsoon season. An inverse relationship was found between agar yield and gel strength. A similar relationship was found for Gracilaria cornea [9] and Gracilaria eucheumoides [19]. Agar gel strengths commonly have been reported as higher from plants harvested during summer or at the time of peak plant abundance, with the difference caused by lower levels of sulfation [30, 31]. In contrast, there also have been reports of lower gel strengths and higher sulfation levels during summer or in high temperatures [7, 8, 32].

Fig. 3. Seasonal variation in gel strength of agar from Gracilaria corticata

The gelling and melting temperatures ranged from 350C to 38.50C and from 56.50C to 70.50C respectively. Maximum melting temperature (70.50C) was found in post monsoon season followed by premonsoon season (620C). Maximum melting temperature coincided with the time of highest gel strength. Gel melting temperature is dependent on molecular weight distribution [33]. Minimum melting temperature (56.50C) was found in monsoon season. This was similar to earlier observation made by Freile-Pelegrin and Robeldo [9]. Gelling temperature of agar showed little variation. It was ranged between 350C and 38.50C. Maximum gelling temperature of 38.50C was recorded in the premonsoon followed by the monsoon season (36.50C). Minimum gelling temperature of 350C was recorded in the postmonsoon season.

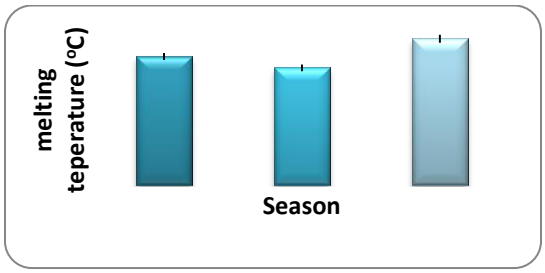

Fig. 4. Seasonal variation in melting temperature of agar from Gracilaria corticata

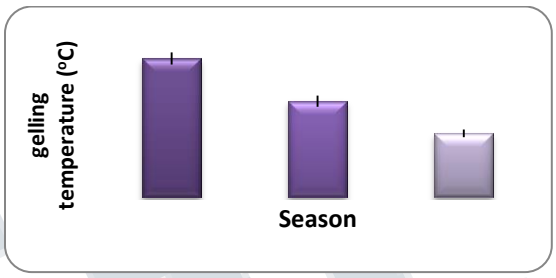

Fig. 5. Seasonal variation in gelling temperature of agar from Gracilaria corticata

The content of 3,6-anhydrogalactose ranged between 20.1% and 24.6% (Fig.6). Maximum 3,6-anhydrogalactose content of 24.6% was observed in monsoon season followed by 23.3% in premonsoon and 20.1% in post monsoon season. Increasing levels of the anhydro sugar result in higher gel strengths of algal extracts [34].

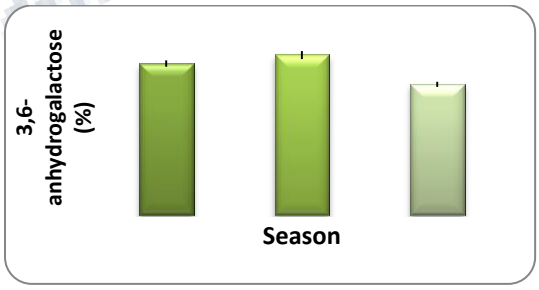

Fig. 6. Seasonal variation in 3,6-anhydrogalactose content from Gracilaria corticata

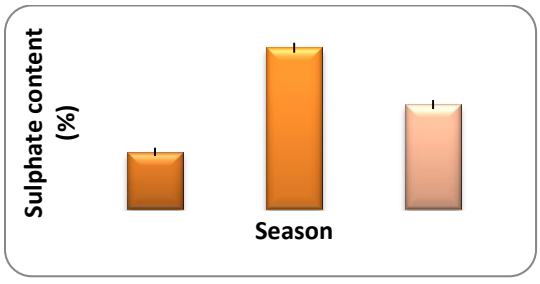

Fig. 7. Seasonal variation in sulphate content of agar from Gracilaria corticata

Lower level of sulphate content increased gel strength of agar. In the present study, the content of sulphate ranged between 1.8% and 5.1% (Fig.7). It was maximum in monsoon season (5.1%) followed by post monsoon (3.3%) and premonsoon (1.8%). The results of this study indicate clear seasonal variations in agar yield, gelling properties and chemical composition of agar from Gracilaria corticata.

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