

## Improvement of yield and quality of agar from *Gracilaria edulis* (Gmelin) Silva

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### ABSTRACT

Optimum levels of alkali/acid and thermal manipulations during extraction of agar were determined to increase the yield and quality of agar from red seaweed *Gracilaria edulis* (Gmelin) Silva. Pre-soaking of dry weeds in water for 2 h increased the yield by 11.44%, but did not improve the gel strength. Pre-soaking for 12 h increased gel strength and melting temperature considerably, but not the yield. Pre-soaking of dry weeds in 1.0N NaOH resulted in gel strength of 135 g.cm<sup>-2</sup>. Pre-soaking of dried weeds with 0.5N and 1.0N HCl even at higher temperature showed improvement neither in yield nor in the quality of agar and also resulted in hydrolysis of agar. Pretreatment at 2.0 - 3.0 N NaOH at 80°C for 1 hour to pre-soaked *G.edulis* for 11 h in water proved to be the most ideal and optimum extraction procedure to obtain higher yield (14.16%), maximum gel strength (291g.cm<sup>-2</sup>), lowest sulphate (0.732%) and highest melting point (99°C) of agar.

### Introduction

Agar is the major constituent of cell wall polysaccharide of certain red algae (Rhodophyceae), especially the members of the families Gelidiaceae, Gelidiellaceae and Gracilariaceae (Doty and Santos, 1983; Chennubhotla *et al.*, 1987). Easy availability of wild *Gracilaria* spp has led to the principal source of agar world wide (Critchley, 1993). Approximately 60% of the world's present production is derived from *Gracilaria* spp (Durairatnam, 1987). Generally, *Gracilaria* spp. yields low quality agar due to high sulphate content and therefore they are called 'agaroids' or 'Gracilaria gum'

(Craigie, 1990). It is well known that quantity and quality of phycocolloid varies not only among species (Cote and Hanisak, 1986), but also due to influence of environmental factors (Craigie *et al.*, 1984), seasonal variations (Oza, 1978; Lahaye and Yaphe, 1988; Freile-Pelegrin and Robledo, 1997; Freile-Pelegrin *et al.*, 1999) and extraction methods (Craigie and Leigh, 1978; Armisen and Galatas, 1987). 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. In the present study, an attempt was made to increase the yield and improve the quality of agar in *Gracilaria edulis* by chemical and thermal manipulations.

### Materials and Methods

Entire plants of *Gracilaria edulis* (Gmelin) Silva growing on coral stones or seagrasses were hand picked during low tide period at Mandapam (78° 8'E, 9° 17'N) coast of Tamil Nadu. The plants were washed thoroughly in running tap water until they were free from sand, other epiphytes and dirt particles. The washed and cleaned material was dried in the sunlight for five days until the material was completely dried.

#### Soaking

To study the effect of soaking period, 20 g of dried and cleaned *G. edulis* was immersed in 400 ml of potable water (1:20 w/v). Time periods such as 2.0 h, 4.0 h, 8.0h and 12.0 h were selected. Control samples were boiled directly.

#### Alkali/Acid pretreatment

To determine the effect of alkali or acid soaking, similarly samples were soaked in dilute solution of HCl / NaOH at concentration of 0.5N - 3.0N for 1h prior to extraction. Presoaked samples in potable water for 11h were transferred to appropriate concentrations of NaOH / HCl for one hour in a water bath at 80°C ± 2. To this mixture 10 ml of 10% CaCl<sub>2</sub>.2H<sub>2</sub>O was added (Baricuatro, 1997) to reduce the loss of agar while processing.

#### Agar extraction

Plants treated with alkali / acid were washed with running tap water to

remove traces of NaOH/HCl. The samples (20 g dry) were boiled in 1litre beaker after adding 400ml distilled water and adjusting the pH to 6.3-6.5 in an autoclave at 1Kg.cm<sup>-2</sup> pressure for 2h (Freile-Pelegrin and Robledo, 1997; Kaliaperumal *et al.*, 2002). The hot extract was recovered after filtration through muslin cloth and pressed in an expeller. The residue was re-extracted with 100ml of hot (85-90°C) distilled water. The filtrates were pooled together and allowed to gel at room temperature, frozen at -20°C for 12 h. The frozen gel was allowed for thawing. The thawed materials were placed on net screens kept slanting for sun drying for 3 days. Agar samples after complete drying were weighed accurately to calculate percentage yield of agar (dried basis).

#### Gel strength

To determine the gel strength and melting point, 1.5% (w/v) agar solution was used. The solution was heated in a water bath. After dissolving agar completely in water, the solution was allowed to form gel at room temperature for 12 h. Gelometer (Funaki and Kojima, 1951) was placed gently on the surface of the gel so that the cylindrical plunger (1cm<sup>2</sup> cross - section) at bottom touched perfectly on gel. Weights were added gradually on the pan of gelometer until it broke the gel gently in 20 seconds. The weight was noted and taken as gel strength and expressed in g.cm<sup>-2</sup>.

The agar solution (1.5% w/v) was kept in hot water bath. The solution was stirred using glass rod while boiling. The molten agar solution was allowed to cool at room temperature. After the gel formation, the gel was again kept in hot water bath. Thermometer bulb was placed in center of the gel and then the

temperature was raised up to 50°C. Spherical glass beads (3mm  $\phi$ ) were placed on the top of the gel. The temperature at which the glass beads started sinking to the bottom was noted as melting temperature of the agar (Whyte *et al.*, 1981).

#### Determination of sulphate content

Sulphate percentage in agar samples was determined spectrophotometrically according to the method of Ji Minghou (1990). A standard sulphate solution was prepared by dissolving 0.1458g of Na<sub>2</sub>SO<sub>4</sub> in 1L of distilled water. Standard graph was plotted and the sulphate content in the agar samples was calculated from the graph.

#### Statistical Analysis

All the extractions, physical and chemical analysis were replicated for five times. Whenever sample quantity was very less for analysis, pooled samples of replications were used for analysis. Data were processed for statistical analysis by the One - way analysis of variance (ANOVA) and significant difference were calculated using the SPSS/PC and MS EXCEL software. Cd values were calculated for finding out which treatment was suitable.

## Results

### Effects of pre-soaking on yield and quality of agar

Agar yield from *Gracilaria edulis* was varying between 10.10 - 11.61 % at different periods of pre-soaking in potable water (Table 1). Agar yield increased slightly (11.44%) when pre-soaking period was limited to 2 h and agar yield reduced (10.10%) when pre-soaking period was prolonged to 12 h. Although agar yield in control (non- soaked) plants showed 11.61%, the gel strength obtained was lower than that of the pre-soaked treatments (54 g.cm<sup>-2</sup>).

For different pre-soaking periods, gel strength varied between 54 - 90 g.cm<sup>-2</sup>. Gel strength of agar improved (90 g.cm<sup>-2</sup>) when presoaked in potable water for 12 h. Agar yield and gel strength were significant at different pre-soaking periods ( $P < 0.05$ ). ANOVA results showed that 2h presoaking in potable water was preferable for agar yield and 12 h treatment preferable for better gel strength. Agar yield and gel strength were negatively correlated ( $r = -0.8898$ ).

Table 1. Effect of pre-soaking in potable water on yield and quality of agar from *Gracilaria edulis* (Mean  $\pm$  S.D.)

Treatment	Yield (%)	Gel strength (g.cm <sup>-2</sup> 1.5% agar [w/v])	Sulphate content (%)	Melting point (°C)
Control	11.612 $\pm$ 0.25	54.0 $\pm$ 3.36	4.944 $\pm$ 0.05	66 $\pm$ 4.14
2 hrs.	11.440 $\pm$ 0.52	61.0 $\pm$ 7.36	4.860 $\pm$ 0.06	70 $\pm$ 4.27
4 hrs.	10.492 $\pm$ 0.32	65.0 $\pm$ 3.80	4.128 $\pm$ 0.06	75 $\pm$ 8.50
8 hrs.	10.274 $\pm$ 0.40	77.0 $\pm$ 5.26	3.846 $\pm$ 0.05	75 $\pm$ 6.80
12 hrs.	10.100 $\pm$ 0.26	90.0 $\pm$ 8.06	3.534 $\pm$ 0.08	77 $\pm$ 1.14

Sulphate content in agar ranged from 3.534 - 4.944%. Sulphate was minimum (3.534%) in 12 h pre-soaking period (Table 1). Melting temperatures of agar were in the range of 66 - 77°C for different pre-soaking periods. Melting point was maximum (77 °C) for agars obtained from *G. edulis* with 12 h period of pre-soaking (Table 1). Correlation between sulphate content and melting point of agar obtained from various pre-soaking treatment were negatively ( $r = -0.9449$ ) correlated. Melting point and sulphate content were significant ( $P < 0.05$ ) at different presoaking periods. The results of ANOVA showed that 12h pre-soaking treatment was the best for sulphate reduction and agar with high melting point ( $P < 0.05$ ).

**Effects of pre-soaking in different concentrations of HCl and NaOH on yield and quality of agar**

When *G. edulis* was presoaked in 0.5 N HCl, the yield from *G. edulis* registered maximum (15.80%). When concentration of HCl was increased for pre-soaking, yield of agar decreased to 4.30% and the gel strength of agar remained lowest (21-22 g.cm<sup>-2</sup>). Sulphate content was minimum (4.18%) in 1.0N HCl pre-soaked sample (Table 2). However,

pre-soaking of dry *G. edulis* in different concentrations of NaOH solution registered higher gel strength of agar that ranged from 125-135 g.cm<sup>-2</sup> (Table 2). Gel strength was maximum in 1.0N NaOH (135 g.cm<sup>-2</sup>). Agar yield and gel strength were negatively correlated ( $r = -0.971$ ). ANOVA results showed that yield and gel strength were significant ( $P < 0.05$ ) in different concentrations of NaOH presoaking. Sulphate content in agar was in the range of 3.644 - 4.796 %, minimum being 3.644% in 1.0N NaOH presoaked samples.

Alkali presoaking of *G. edulis* had improved melting point (82°C) of agar (Table 2). Sulphate content was showing significant ( $P < 0.05$ ) reduction with increasing concentrations of NaOH pre-soaking. ANOVA results indicated that 1.0N NaOH pre-soaking was ideal for sulphate reduction. Sulphate content and melting point were negatively correlated ( $r = -0.9983$ ).

**Effect of alkali/ acid pre-treatment with raising temperature for 1 h on the yield and quality of agar**

At different concentrations of NaOH pre-treatment and increased temperature (80°C), the yield of agar ranged from 10.15 - 14.15% ( $P < 0.05$ ). Yield of

Table 2. Effect of pre-soaking in different concentrations of HCl and NaOH on yield and quality of agar from *Gracilaria edulis* (Mean  $\pm$  S.D.)

Treatment	Yield (%)	Gel strength (g.cm <sup>-2</sup> 1.5% agar [w/v])	Sulphate Content (%)	Melting point (°C)
Potable Water	12.364 $\pm$ 0.13	60.0 $\pm$ 4.40	4.710 $\pm$ 0.06	70 $\pm$ 3.14
0.5 N HCl	15.844 $\pm$ 0.37	22.0 $\pm$ 1.60	4.602 $\pm$ 0.12	NIL
1.0 N HCl	4.376 $\pm$ 0.22	21.0 $\pm$ 1.09	4.180 $\pm$ 0.04	NIL
0.5 N NaOH	12.400 $\pm$ 0.15	125.0 $\pm$ 2.07	3.759 $\pm$ 0.03	80 $\pm$ 2.40
1.0 N NaOH	12.124 $\pm$ 0.04	135.0 $\pm$ 2.24	3.644 $\pm$ 0.02	82 $\pm$ 1.64

agar increased (14.15%) in 2.0 N NaOH and reduced considerably (10.15%) in 3.0N NaOH (Table 3). ANOVA results showed that 2.0N NaOH pre-treatment at 80°C was ideal for agar yield. Gel strength varied from 69 - 291 g.cm<sup>-2</sup>. Pre-treatment of 3.0 N NaOH at 80°C increased gel strength to 291 g.cm<sup>-2</sup> (Table 3) from the minimum of 168 g.cm<sup>-2</sup> in 0.5N NaOH pretreatment, which was 240% higher than the gel strength achieved by control. Gel strength showed significant variation ( $P < 0.05$ ) at different treatments. Agar yield and gel strength were negatively correlated ( $r = -0.0404$ ). Sulphate content varied from 0.732 % - 5.548% with the lower values in higher levels of NaOH pre-treatment at 80°C.

Melting point at different concentrations of NaOH pre-treatment at 80°C temperature ranged from 68 - 99°C (Table 3) with the maximum in 3.0N NaOH pretreatment (99°C). However, different concentrations of HCl pretreatment (0.5N - 3.0N) at 80°C temperature resulted hydrolysis of agar and hence could not be extracted.

ANOVA results showed that 2.0N NaOH pretreatment at 80°C was highly preferable for quality (gel strength) and yield improvement ( $P < 0.05$ ; Cd = -0.057 (agar yield); Cd = 3.305 (gel strength); Cd = 0.048 (sulphate); Cd = 2.075 (melting point).

### Discussion

Yield, gel strength, melting temperature and sulphate content of agar from *Gracilaria* spp are decided by a number of factors such as growing season, growth stages, location and among species (Ji Minghou, 1990; Whyte *et al.*, 1981) temperature and salinity (Luhan, 1992; Sasikumar *et al.*, 1999) and nitrate levels in water (Christeller and Laing, 1989). In the present study, pre-soaking of dry weeds in water for 2 h increased the yield to 11.44% but did not improve the gel strength of agar. Presoaking in water for 2h increased the yield may be due to water soluble non-agar materials and algal debris in the extraction (Haneefa Koya, 2000). On the otherhand presoaking in water for 12 h duration increased considerably gel strength, melting temperature and reduced

Table 3. Effect of alkali/acid pre-treatment with raising temperature for one hour before extraction on the yield and quality of agar (Mean  $\pm$  S.D.)

Treatment	Yield (%)	Gel strength (g.cm <sup>-2</sup> 1.5% agar [w/v])	Sulphate Content (%)	Melting point (°C)
Control (0.0 N NaOH/HCl)	11.772 $\pm$ 0.03	69 $\pm$ 1.80	5.548 $\pm$ 0.07	68 $\pm$ 2.70
0.5 N NaOH	13.143 $\pm$ 0.05	168 $\pm$ 1.51	4.208 $\pm$ 0.01	88 $\pm$ 2.72
1.0 N NaOH	13.226 $\pm$ 0.05	191 $\pm$ 1.92	3.556 $\pm$ 0.05	95 $\pm$ 0.83
2.0 N NaOH	14.156 $\pm$ 0.06	274 $\pm$ 2.73	0.844 $\pm$ 0.03	96 $\pm$ 1.30
3.0 N NaOH	10.156 $\pm$ 0.05	291 $\pm$ 5.45	0.732 $\pm$ 0.02	99 $\pm$ 1.00
0.5 N HCl	NIL	NIL	NIL	NIL
1.0 N HCl	NIL	NIL	NIL	NIL
2.0 N HCl	NIL	NIL	NIL	NIL
3.0 N HCl	NIL	NIL	NIL	NIL

sulphate content (Table 1). The reason may be attributed to the removal of impurities during prolonged soaking, including some water-soluble inhibitors such as mud particles, which might affect adversely the agar quality. The results are correlating with earlier reports (Thomas and Krishnamurthy, 1976; Kaliaperumal *et al.*, 1987) on gelstrength (70-123 g.cm<sup>-2</sup>). Pre-soaking of dry weeds in 1.0 N NaOH solution is preferred over pre-soaking with dilute HCl (Table 2), to achieve the increased gel strength (135 g.cm<sup>-2</sup>), reduced sulphate content (3.64%), higher melting temperature (82°C) in agar but not for improvement in the yield.

These findings are agreeable with earlier reports by Ji Minghou (1990) and Coppen (1991) on *Gracilaria* spp and by Freile-Pelegrin and Robledo *et al.* (1997) on *Gracilaria cornea* who advocate the need for alkali pretreatment to improve the quality of agar. In the present study, the treatment of presoaked *G.edulis* with 2.0-3.0 N NaOH at 80°C for 1 h proved ideal and an optimized chemical manipulation in extraction method to obtain moderately high yield (14.156%) and maximum gel strength (291g.cm<sup>-2</sup>), lowest levels of sulphate (0.732 %) and highest melting temperature (99°C) of agar (Table 3). Our results are well identical with previous results obtained for *Gracilaria blodgettii* and *G.verrucosa* in 2.0 N NaOH pretreatment (Sasikumar *et al.*, 1997) and *Gelidiella acerosa* treated with 0.5 N NaOH treated sample (Mathew *et al.*, 1993), in *Gracilaria cornea* from Yucatan, Mexico (Freile-Pelegrin and Robledo, 1997), *Gracilaria edulis* (Durairatnam, 1987), *Gracilaria sjoestedtii* and *G.verrucosa* (Craigie *et al.*, 1984), *Gracilariopsis heteroclada* (Baricuatro, 1997). The results obtained in 2.0 - 3.0N NaOH at 80°C treatment are meeting the current

requirement in international food market on agar, which is sulphate content less than 4% (Armisen, 1995). The melting temperature (99°C) and gelstrength improvement (291 g.cm<sup>-2</sup>) are reaching the requirements (>85°C) of Committee on codex specification (1981) and Japanese grade-2 agar (gel strength above 220 g.cm<sup>-2</sup>) (Ji Minghou *et al.*, 1985).

When the agar industries in India are contacted for any requirement of technological innovations using pretested interview schedules (Kaladharan and Pillai, 1998) majority of them responded for new technology to improve the quality of agar. Hence, this study may be of immense help to the agar industries in India.

From the above experiments it can be surmised that pre-treatment of presoaked *G. edulis* with 2.0 - 3.0 N NaOH solution for 1 hr at 80°C is most suitable for obtaining optimum yield of agar with desirable qualities. This information may be recommended for the agar industries after conducting few more multi seasonal trials.

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