Influence of post-harvest treatment on shelf life and agar quality in seaweeds

*Gracilaria edulis*(Rhodophyta/Gigartinales and *Gelidiella acerosa*(Rhodophyta/Gelidiales)

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The effect of acetic acid and alkali (KOH) pretreatments on the shelf life of *Gracilaria edulis* and *Gelidiella acerosa* were studied with respect to the agar yield and physical properties. Algae collected from the Gulf of Mannar, southeast coast of India were treated with 1) 0.5% acetic acid for 1 hr, 2) 1% KOH for 1 hr and 3) 0.5% acetic acid 1 hr followed by neutralization in 1% KOH for 1 hour. *Gracilaria edulis* pretreated with acid + alkali gave the maximum agar yield (69.2 ± 12.3%), gelling temperature (50.5 ± 1°C) and melting temperature (82.5 ± 1.5°C). However no improvement in gel strength was recorded over control. Acid treated *Gelidiella acerosa* yielded the maximum agar (59.2 ± 3.9%) while exhibiting a gel strength of 295.1 ± 18.6 g.cm⁻², a gelling temperature of 53 ± 2.5°C and a melting temperature of 88.7 ± 0.7°C. When the pretreated algae were stored for 4 months, for *G. edulis* agar yield remained same but its physical properties decreased gradually. In *G. acerosa* agar yield and melting temperature were constant, but gel strength and gelling temperature decreased drastically. It is suggested that agar industry can treat *Gelidiella acerosa* with acetic acid and *Gracilaria edulis* with acetic acid and KOH prior to storage. This will help to increase the agar yield and its physical properties on immediate use and maintain agar yield on long term use.

**[Key words]:** Agar yield, gel strength, gelling temperature, melting temperature, acid, alkali, treatment, storage

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Agars are polysaccharides within the intracellular matrix of members of marine red algal families, Gracilariaeae and Gelidiellacaeae. The yield and physical properties of agar such as gel strength, gelling and melting temperature define its value to industry. Generally, *Gracilaria* spp. produce agar with low quality due to high sulphate content and therefore they are called agaroides. However, transformations of agaroides into real agar can be done by alkali treatment that converts L-galactose-6 sulphate to 3,6 anhydro L-galactose which is responsible for the enhancement of gel forming ability. Acid treatment applied to the species *Gelidiella acerosa* and *Gracilaria lamaneiformis* improved gel quality. Combination of alkali at different concentrations, together with different temperatures and durations of treatment has been applied to determine best treatment for certain agar yielding seaweeds. *Gelidiella acerosa* (Forsskaal) Feldmann et Hamel (Rhodophyta/Gelidiales) and *Gracilaria edulis* (Gmelin) Silva (Rhodophyta/Gigartinales) are important sources of raw material that are utilized for agar production in India. Food grade agar is produced from *Gracilaria edulis* and bacteriological grade from *Gelidiella acerosa* and required for entire seaweed industries in India is met by harvesting from natural beds along Tamilnadu coast. These algae are processed by industry during the peak production period from September to February. Raw materials are stored until they are utilized. Unless seaweed is quickly and thoroughly dried and kept dry, phycocolloids are irreversibly degraded both by catabolic enzymes and micro organisms. Bacteria as well as fungi have been implicated in the degradation of phycocolloid during storage. No study has been reported on the effect of

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acid or alkali treatment on shelf life of algae. Present study was carried out with the aim of determining whether the shelf life and agar quality of *G. acerosa* and *G. edulis* could be extended by the use of post-harvest treatments.

**Materials and Methods**

*Gracilaria edulis* and *Gelidiella acerosa* samples were collected from sub tidal regions of Kurasadai island and Sethukarai along the Gulf of Mannar (lat. 8°35’ to 9°25’N; long. 78°08’ to 79° 30’E), southeast coast of India during the peak growth season of November 1997. The harvested materials were immediately transported to the laboratory where they were thoroughly washed with seawater.

The pre-extraction treatment involved a) soaking of seaweed samples in 0.5% acetic acid at 25-27 °C and *pH* 8.5 for 1 hr, b) alkali treatment with 1% KOH for 1 hr at 88-90° C and *pH* 8.5, c) alkali treatment (1% KOH ) for 1 hr followed by neutralization in 0.5% acetic acid for 1 hr. The treatment chemicals were drawn from 25 litres batches of 0.5% acetic acid and 1% KOH solutions that were prepared in separate glass tanks of 50 litres capacity. The algae (2.5 kg fresh weight) were dipped in the solutions for 1 hr in order to achieve complete absorption. After acid and alkali treatment, the plants were washed in running water to remove excess acid or alkali, sun dried, labeled and stored in sealed plastic bags until agar extraction. Control samples (without treatment) were directly washed in seawater, dried, labeled and stored in sealed plastic bags along with treated samples. Agar yield and its physical properties viz. gel strength, gelling and melting temperature were estimated for the above stored samples for every 15 days up through a four month period (120 days).

About 20 g of seaweed powder was added to 400 ml of distilled water and the samples were boiled under steam pressure at 15 pa for 2-3 hr. The extract was pressure filtered into a small tray and the filtrate was cooled at room temperature until gelation occurred. The gel was frozen overnight and thawed on the following day. The thawed agar was oven dried for 24 hr at 60°C, cooled and weighed to calculate percentage agar yield. Extraction and physical agar analysis were performed in six replicates. Dried agar was ground in a commercial blender and reconstituted in 1.5 % w/v solutions with double distilled water to measure the physical properties viz. gel strength, gelling and melting temperature.

The hot solutions (100 ml) of 1.5% (w/v) agar and glass bead (5 mm diameter) were placed in glass beakers (7 cm height and 4.5 cm diameter) and cooled at room temperature (27°C). The beaker was tilted up and down until the glass bead ceased moving. The gel temperature in the beaker was immediately measured by inserting a thermometer (0.1°C). Gel strength was measured after the gels had set overnight at room temperature (27°C) by measuring the weight of mercury bead (g.cm²) causing a cylindrical plunger (1cm² cross section) to break the gel⁹. Melting temperature of the gel in the beaker was measured by placing an iron bead (9 mm diameter) on the gel surface. The beaker was kept in a water bath and heated (100°C). The melting point was recorded with a thermometer (0.1°C) when bead sank into the solution.

Data were subjected to Pearson’s product moment correlation test to determine the linear relationship between agar properties during storage periods. The significance of differences in yield and physical properties between pre extraction treatments were tested using analysis of variance (ANOVA).

**Results**

Variations in agar yield and physical properties of *Gracilaria edulis* and *Gelidiella acerosa* in all treatments are shown in Figs. 1 and 2. The agar content of *G. edulis* ranged from 18.5 to 50.3% in untreated alga (Fig. 1A). When acid pretreatment was given, the agar yield varied from 30.5% to 60.8%. The treatment with 1% KOH produced an agar yield from 34.0% to 60.6%. For 0.5% acetic acid + 1% KOH treatment, the agar yield ranged from 55.3% to maximum of 69.2%. The maximum yield value (69.2%) was found in acid + alkaline treated alga on the 60th day of storage. By this treatment a high agar yield (58.2%) was obtained even after the sample had been stored for 120 days. In the case of both acid and alkali treatments, the agar yield decreased from initial period up to 75 days. Thereafter, the yield increased till 120 days. A highly significant correlation (P<0.001) was observed between these two treatments. Agar yield showed no variation during storage but significant variations (p<0.01) among three treatments (Table 1).

In *Gelidiella acerosa*, agar yield varied from 16.2% to 47.8% in untreated sample, 33.0% to 59.2% in acid treated alga, 9.2% to 28.1% in alkali treated alga and 16.5% to 56.3% in acid +alkali treatment. Maximum agar yield (59.2%) was found in acetic acid
Fig. 1—Effect of pre-extraction treatment on agar yield of— A) Gracilaria edulis and B) Gelidiella acerosa and gel strength of C) Gracilaria edulis and D) Gelidiella acerosa.
Fig. 2—Effect of pre-extraction treatment on gelling temperature of A) *Gracilaria edulis* and B) *Gelidiella acerosa* and melting temperature of C) *Gracilaria edulis* and D) *Gelidiella acerosa*
G. edulis. For the treatment with 0.5% acetic acid, gel strength varied from 129.2 g. cm\(^{-2}\) to 231.3 g. cm\(^{-2}\). Maximum (295.1 g. cm\(^{-2}\)) and minimum gel strength (26.2 g. cm\(^{-2}\)) were recorded in acid treated samples (90 days and zero day storage respectively). Significant variation in gel strength during storage period (p<0.05) and no variation between the three treatments were observed (Table 1).

The gelling temperature of Gracilaria edulis ranged from 41.2°C to 50°C in control sample, 40.0°C to 45.7°C in acid treated sample, 39°C to 47°C in alkali treated alga and 41°C to 50.5°C in acid + alkali treated sample (Fig.2A). Acetic acid + KOH treatment gave highest gelling temperature (50.5°C) on the 15\(^{th}\) day of storage and only KOH treatment produced gel with lowest temperature (39°C) on 120\(^{th}\) day. Significant variations (p<0.05) among the three pretreatments and also during storage period (p<0.01) were observed (Table 1).

In Gelidiella acerosa, gelling temperature ranged from 38.2°C to 52.0°C in untreated alga, 38.0°C to 53°C in acid treated alga, 38.0°C to 49.5°C in alkali treated alga and 38.2°C to 52.5°C in acid + alkali treated alga (Fig.2B). Pretreatment with 0.5% acetic acid produced highest gelling temperature (53°C) while treatment with 1% KOH gave lowest gel temperature (38.0°C) on 60 days. Significant differences during storage period (p<0.01) and no significant differences between the three pretreatments were observed (Table 1). Gel temperatures were drastically declined in stored samples up to 60 days and then slowly increased until 120 days. This trend was observed in both untreated and treated alga. A highly significant positive correlation (P<0.01) was observed between acid + alkali treated samples of G. acerosa and G. edulis and same trend (P<0.05) was also observed between control (untreated) samples of G. acerosa and G. edulis.

The melting temperature of Gracilaria edulis agar gels ranged from 66.0°C to 82.2°C in untreated alga, 63.0°C to 80.0°C in acid treated sample, 66.0°C to 79.0°C in alkali treated alga and 70.0°C to 82.5°C in

### Table 1—Analysis of variance on the yield and physical properties of agar from G. edulis and G. acerosa treated with acid and alkali

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pretreatment on the 120\(^{th}\) day and minimum yield (9.2%) was recorded in alkali (KOH) treatment on the 90\(^{th}\) day (Fig. 1B). When G. acerosa was pretreated with acetic acid, the agar yield increased exponentially until the end of the storage period. For treatment with acid+alkali, yield increased until 90 days of storage and then decreased. A reverse trend (P<0.01) was observed in alkali treatment where yield decreased until 90 days of storage and then increased. ANOVA showed no variation during storage but significant variations (p<0.01) between the three treatments (Table 1). During the storage period, alkali pretreatment for G. edulis and G. acerosa showed a significant positive correlation (P<0.01).

Gel strength in Gracilaria edulis varied from 20.7 to 96.5 g. cm\(^{-2}\) in untreated alga, 13.5 to 58.7 g. cm\(^{-2}\) in acetic acid treatment, 12.0 to 81.4 g. cm\(^{-2}\) in alkaline treatment and 21.9 to 86.6 g. cm\(^{-2}\) in acid+alkali treatment (Fig. 1C). The maximum gel strength (96.5 ± 2.9 g. cm\(^{-2}\)) was recorded in untreated alga on the 15\(^{th}\) day of storage. Minimum gel strength (12.0 g. cm\(^{-2}\)) was found in alkali treatment on the 30\(^{th}\) day. Significant variations (p<0.01) in gel strength between the three treatments and during storage period (p<0.05) were observed.

In Gelidiella acerosa (Fig. 1D), gel strength ranged from 106 g. cm\(^{-2}\) to 258 g. cm\(^{-2}\) in untreated alga. For the treatment with 0.5% acetic acid, gel strength ranged from 26.2 g. cm\(^{-2}\) to 295.1 g. cm\(^{-2}\). In 1% KOH treatment, it varied from 38.4 g. cm\(^{-2}\) to 201.2 g. cm\(^{-2}\). When G. acerosa is pretreated with acid + alkali, gel strength varied from 129.2 g. cm\(^{-2}\) to 231.3 g. cm\(^{-2}\). Maximum (295.1 g. cm\(^{-2}\)) and minimum gel strength (26.2 g. cm\(^{-2}\)) were recorded in acid treated samples (90 days and zero day storage respectively). Significant variation in gel strength during storage period (p<0.05) and no variation between the three treatments were observed (Table 1).
acid +alkali treated alga (Fig. 2C). The maximum melting temperature (82.5°C) was recorded in acid + alkali pre treated alga, whereas minimum melting temperature (63.0°C) was observed in acetic acid treated alga on the 90th day of storage. ANOVA showed significant differences (p<0.01) during storage and between three treatments.

The melting temperature of Gelidiella acerosa agar gels ranged from 80.0°C to 84.5°C in control alga, 55.5°C to 88.7°C in acetic acid treated alga, 70.0°C to 85.5°C in alkali treated alga and 70.0°C to 85.0°C in acid + alkali treated alga (Fig. 2D). Acetic acid pretreatment gave highest (88.7°C) as well as lowest (55.5°C) melting temperature on the 15th and 90th day of storage respectively. ANOVA showed significant differences (p<0.01) during storage period and no difference between the three treatments (Table 1). In alkali treated alga and acid + alkali treated alga, melting temperature decreased until 75 days of storage and then increased until 120 days. A highly significant correlation (P<0.001) was observed between these two treatments.

Discussion
Preextraction treatment using alkali or acid was observed to facilitate the retention of a high agar yield as compared to untreated samples in Gracilaria edulis and Gelidiella acerosa. Acid + alkali pretreatment enhanced the agar yield in G. edulis, while acid treatment yielded higher agar in G. acerosa. Similar higher agar yields have been observed in acid treated G.acerosa from Philippines coast. In G. acerosa pretreatment enhanced agar after 120 days storage as compared to 15 days storage. In G. edulis, after 120 days storage yield is close to 15 days storage. It has also been reported that agar content decreased during storage because of hydrolysis due to microbial or enzymatic degradation. Armisen reported that in Gracilaria chilensis grown in temperate water, hydrolysis was detected after 6-8 months of storage, but in tropical water Gracilaria species hydrolysis occurred within a few months. Jafferey et al. reported in Gracilaria gracilis, epiphytic bacteria exploit agar as a nutrient. Mow Robinson & Rheinheimer and Rieper-Kirchner found bacteria specialized in polysaccharide decomposition abound in areas where relevant macroalgae prevailed. Kong & Chan isolated agar digesting bacteria from the red algae Polysiphonia lanosa (Linnaeus) Tandy and Hypnea chariodes Lamouroux. It is postulated that when algae were treated with alkali or acid prior to storage the destruction of microbial contamination and subsequent inactivation of enzymes takes place. Therefore, good agar yields were obtained even after 120 days of storage. In addition to microbial destruction, acid treatment has been postulated to facilitate the extraction of polysaccharide by disrupting cross links occurring in the algal structure. The significant correlation (P< 0.001) between alkali treated G. acerosa and G. edulis indicated that KOH induces similar trends in both algae during storage periods.

The agar content found in acid+alkali treated Gracilaria edulis was higher than in other Gracilaria species such as G.edulis(21.8%) from Brazil, G.cervicornis (21%) from Venezuela and G.cornea from Mexico(22.1%). Similarly, agar yields of acid treated G.acerosa in the present study were higher than the yields observed for G. acerosa (12.±2.5%; 19.7±3.4) from the Philippine coast.

The gel strength of Gelidiella acerosa attained its maximum among acid pretreated algae. This coincides with results of G.acerosa from the Philippines coast. Although acid treatment enhances the gel strength initially, it has no impact on shelf life as gel strength reduced drastically and reached its lowest value on 120th day of storage. On contrary to G. acerosa, the gel strength was lower in treated alga than untreated alga in the case of G. edulis, and lowest on 120th day of storage as compared to initial period.

During the present study relatively higher gelling temperature was observed in alkali + acid treated agar of G.edulis and acid treated agar of G. acerosa. However, treatment has no effect on shelf life of algae as gel temperature constantly decreased up to 120 days of storage in both algae.

Gracilaria acerosa could be able to retain its melting temperature as the value on 120th day is closure to 15 days. However, in G. edulis, melting temperatures are in decreasing trend till 120 days of storage Analysis of variance (ANOVA) showed significant differences in agar yield and physical properties between three treatments in G. edulis. However, in G. acerosa, significant differences between treatments were obtained only for agar yield. No significant difference was observed in the physical properties of agar extracted from such samples. During 120 days storage, significant differences in physical properties of agar were observed in both G.edulis and G.acerosa.
From the present study, it was found that the acetic acid pretreatment given to *G. acerosa* resulted in a high agar yield with corresponding high gel strength, gelling and melting temperatures as compared to agar from untreated alga. Also relative to control samples the alkali + acid pretreatment given to *G. edulis* yielded more agar with high gelling and melting temperatures but no substantial increase in gel strength was observed by this treatment. As far as shelf life is concerned, *G. edulis* could retain its agar yield during storage. However, its physical properties decreased gradually. *Gracilaria acerosa* could retain agar yield and melting temperature while gel strength and gelling temperature decreased drastically.

It is suggested that agar industry can treat *Gelidiella acerosa* with acetic acid and *Gracilaria edulis* with acetic acid and KOH prior to storage. This will help to increase the agar yield and its physical properties on immediate use and maintain agar yield on long term use.

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**Reference**