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Does storage time influence yield and agar properties in the tropical agarophyte *Gracilaria cornea*?

Yolanda Freile-Pelegrín
CINVESTAV-Unidad Mérida, AP 73 Cordemex 97310, Mérida, Yucatán, México
(e-mail freile@kin.cieamer.conacyt.mx)

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Abstract

The agar yield and quality characteristics of *Gracilaria cornea* from Yucatán, Mexico, were studied during 18 months of storage. Biomass was stored at a temperature of 22.1 ± 0.9 °C and humidity of 59.8 ± 3.6%. The agar content varied erratically, but the average value was practically constant over the storage period with an average of 20.1 ± 1.5%. Gel strength, gelling and melting temperatures were negatively affected by the total storage time. No significant changes were found during the first five months for these characteristics with mean values of 1134 ± 57 g cm^{-2}, 40.8 ± 0.4 °C and 91.2 ± 0.9 °C respectively. Agar degradation was evident after the fifth month and accounted for a 17% loss in gel strength and $\sim$ 7% in gelling and melting temperatures. Nevertheless, gel strength values remained around 930 ± 23 g cm^{-2} with no significant changes until the end of the storage period. The decrease in gel strength showed a significant relationship with decrease in 3,6-anhydrogalactose but not variation in sulphate content. This was probably due to agar hydrolysis caused by enzymatic processes of endogenous and/or microbial origin. These results suggest that the tropical *G. cornea* had a similar resistance to degradation during storage to that observed for *G. chilensis*, a cold water species. Agar quality and yield in *G. cornea* after one and a half year of storage are within the range of food grade agar.

Introduction

The genus *Gracilaria* is one of the most important agarophyte resources on the world, mainly due to the availability of biomass and to the discovery of alkali treatment, which can increase the quality of their agars (Critchley, 1993). However, storage of *Gracilaria* suffers the problem that there is irreversible damage of the polysaccharide caused by endogenous enzymes and agarolytic bacteria such as *Bacillus cereus* and *Pseudomonas atlantica* (Armisén, 1991). This occurs after long storage periods, even under favorable conditions (Armisén, 1995). Therefore, different ways to prevent microbial decomposition during storage have been described by Kim (1970) and Armisén (1995): a) preservation in formaldehyde, b) alkali treatment before storage and c) irradiation with γ-radiation from ^{60}Co which acts as a biocide. However, the above mentioned treatments have some disadvantages: a) formaldehyde is highly toxic and causes pollution problems, b) the adjustment of the alkali treatment parameters (temperature, time and alkali concentration) are very important to obtain a good agar quality and so, these parameters must be known previously to the storage, and c) the γ radiations produce a decrease in gel strength and is an expensive process (Armisén, 1995). Thus, these methods are not feasible for agar producers from both, ecological and an economical point of view. Agar degradation is more acute in warm water *Gracilaria* species. In the tropics, the high temperature and humidity could promote the enzymatic degradation during storage, which in turn produce the hydrolysis of the colloid even in a few months. Nevertheless, mariculture sites are bond with field tropical conditions (rain and high temperature) that make ineffective the dry and storage methodology available (Kapraun, 1999).

Nowadays, *Gracilaria* production is dominated by wild and cultivated material from Chile, which together constitute about 73% of the world total (Zemke-White & Ohno, 1999). However, other *Gracilaria* sources for agar production are being considered. Recently, studies on *Gracilaria cornea* J. Agardh from Yucatán (México) have shown good yields and excellent agar quality (Freile-Pelegrín & Robledo, 1997a, 1997b). Moreover, the Yucatán coast has adequate conditions for mariculture purposes where studies on *G. cornea* field cultivation are being done (Robledo, 1999). As for future commercial and industrial development of *G. cornea* in Yucatán, knowing the resistance of this specie to modify its chemical structure is important even under extended storage period. The aim of this study was to evaluate changes in yield and in physico-chemical agar properties during 18 months to determine maximum storage time of *G. cornea* biomass without lost of its quality.

Materials and methods

Gracilaria cornea was harvested at Dzilam de Bravo (21°23′ N, 88°57′ W), north east coast of Yucatán peninsula. The plants were collected by diving at 1–3 m depth during May 1998. The biomass was transported to the laboratory, washed thoroughly with tap water and sun dried. Individual samples of 25–30 g dried material were separated and stored.

Storage conditions and sampling

Drastic changes in temperature and humidity prevail in Yucatán due to its tropical weather. To eliminate these abrupt fluctuations in above mentioned parameters, the bags were placed in plastic boxes under indoor conditions of temperature and humidity. Room temperature and relative humidity were registered every month (in triplicate) before each agar extraction and the humidity of the algal material was obtained as changes in percent dry weight. Three samples of stored material were taken from the storage boxes each month over a period of 18 months for agar analysis.

Agar extraction

Alkali treatment was carried out on 20 g dry biomass. The seaweeds were soaked overnight in 400 mL of 3% NaOH solution at room temperature. This was followed by 3 h in a water bath at 85 °C. To remove excess NaOH, the plants were washed 1 h with running tap water and soaked in 0.025% H_2SO_4 solution for 2 h. To eliminate the acid, samples were washed twice with distilled water. Extraction was done by boiling for 1.30 h in 600 mL distilled water at 6.3–6.5 pH, blended with diatomaceous earth and pressure-filtered. The filtrate was frozen, thawed, oven dried at 60 °C and weighed to obtain agar yield.

Rheological and chemical analysis

A 1.5% w/v solution was prepared to test the gel strength, gelling and melting temperatures. Nikansui Shiki gelometer (1 cm^2 plunger) was used for the gel strength determination. Gelling and melting temperature was recorded with a precision thermometer (0.1 °C divisions) as previously described by Freile-Pelegrín & Robledo (1997a).

The 3,6-anhydrogalactose content (3,6 AG) was determined by the colorometric method of Matsuhiro and Zanlungo (1983) and sulphate content was measured turbidimetrically after hydrolyzing 25 mg agar in a sealed tube for 12 h in 1N HCl at 105 °C (Jackson & McCandless, 1978).

Statistical analyses

Data were tested for normality (Kolmogorov-Smirnov) and subjected to the Bartlett's test for homogeneity of group of variances. All heterogeneous data groups were transformed by different methods including arcsin square root of x, $\log(x+1)$ and $\ln(x+1)$ to produce the homogeneity required. The effect of storage time in agar yield and physico-chemical properties was determined using ANOVA ($p = 0.05$). A Pearson's correlation coefficient was used to determine the correlation between agar properties.

Results

The storage conditions were practically constant throughout the study period. Average room temperature and humidity were 22.1 ± 0.9 °C and 59.8 ± 3.6% respectively. The water content in the plants had a small variation along the 18 months (11.6 ± 0.5%).

Agar content had an erratic behavior and statistical analysis showed a significant difference along the storage period ($p < 0.05$). Nevertheless, the tendency line was practically constant with an average of 20.1 ± 1.5% (Figure 1). Maximum values were obtained for the sixth (23.1%) and the thirteenth (22.9%) month, and the minimum agar yield for the seventeenth month

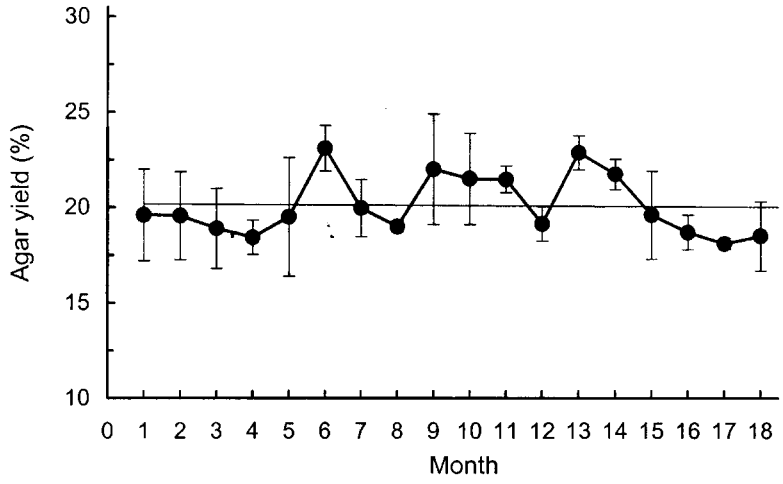

Figure 1. Agar yield along the study period. Bars represent the standard deviation.

(18.1%). On the other hand, the same figure shows that fluctuations occurred at the middle of the storage period, between the sixth month and the fourteenth month with an average of $21.2 \pm 1.4\%$. During the first five and the last four months the yield average was $19.2 \pm 0.5\%$ and $18.7 \pm 0.5\%$ respectively.

Figure 2 shows the quality parameters along the study period. Overall, gel strength, gelling and melting temperatures, and gelation hysteresis were negatively affected by the total storage time ($p < 0.05$). No significant changes were found during the first five months for gel strength ($p > 0.05$) with values ranging between 1073 g cm^{-2} and 1240 g cm^{-2}, $\bar{x}$ = 1134 ± 57 g cm^{-2} (Figure 2A). After the sixth month, the gel strength decreased to values remaining around 930 ± 23 g cm^{-2} without significance changes until the end of the storage period ($p > 0.05$).

In the same way, gelling and melting temperatures, and gelation hysteresis had a similar behavior as gel strength. During the first five months mean values were 40.8 ± 0.4 °C for gelling temperature, 91.2 ± 0.9 °C for melting temperature (Figure 2B), and 50.4 ± 1.1 °C for gelation hysteresis (Figure 2C). A strong decrease was evident after the fifth month for gelling and melting temperature. These values fluctuated between 37.5 and 39.3 °C (38.3 ± 0.6 °C) for gelling temperature, between 82.5 and 88.4 °C (85.1 ± 1.6 °C) for melting temperature (Figure 2B), and between 44.2 and 50.1 °C for hysteresis (46.8 ± 1.8 °C) (Figure 2C). The three parameters were positively correlated with gel strength ($r = 0.74$, $p < 0.01$ for gelling temperature; $r = 0.71$, $p < 0.01$ for melting temperature; $r = 0.50$, $p < 0.01$ for gelation hysteresis).

Sulphate content throughout the 18 months of storage is shown in Figure 3A. The values ranged from a minimum of 1.5% for the 11th, 14th and 15th month to a maximum of 2.8% for the ninth month. Average sulphate content during total storage time was $1.8 \pm 0.3\%$.

The content of 3,6 AG followed a similar pattern to gel strength during the storage time, with no significant differences between the first seven months ($p > 0.05$) with an average of $41.6 \pm 0.2\%$ (Figure 3B). From the eighth month until the end of study 3,6 AG decreased to an average of $39.0 \pm 0.7\%$ without any significant changes ($p > 0.05$). A positive correlation was found between 3,6 AG and gel strength ($r = 0.59$, $p < 0.01$), gelling temperature ($r = 0.44$, $p < 0.01$), melting temperature ($r = 0.59$, $p < 0.01$) and gelation hysteresis ($r = 0.52$; $p < 0.01$).

Discussion

The quality of *G. cornea* agar was modified by storage time (18 months) under the conditions used for this study. However, agar content was practically constant along storage period with values in the range or exceeding those obtained for commercial agarophytes (Table 1). Moreover, agar yield had a small increase during the first six months. This could be related to a partial maceration of cell walls that allowed a better extraction process. The same effect has been described in *Gelidium* where biomass with a moisture content

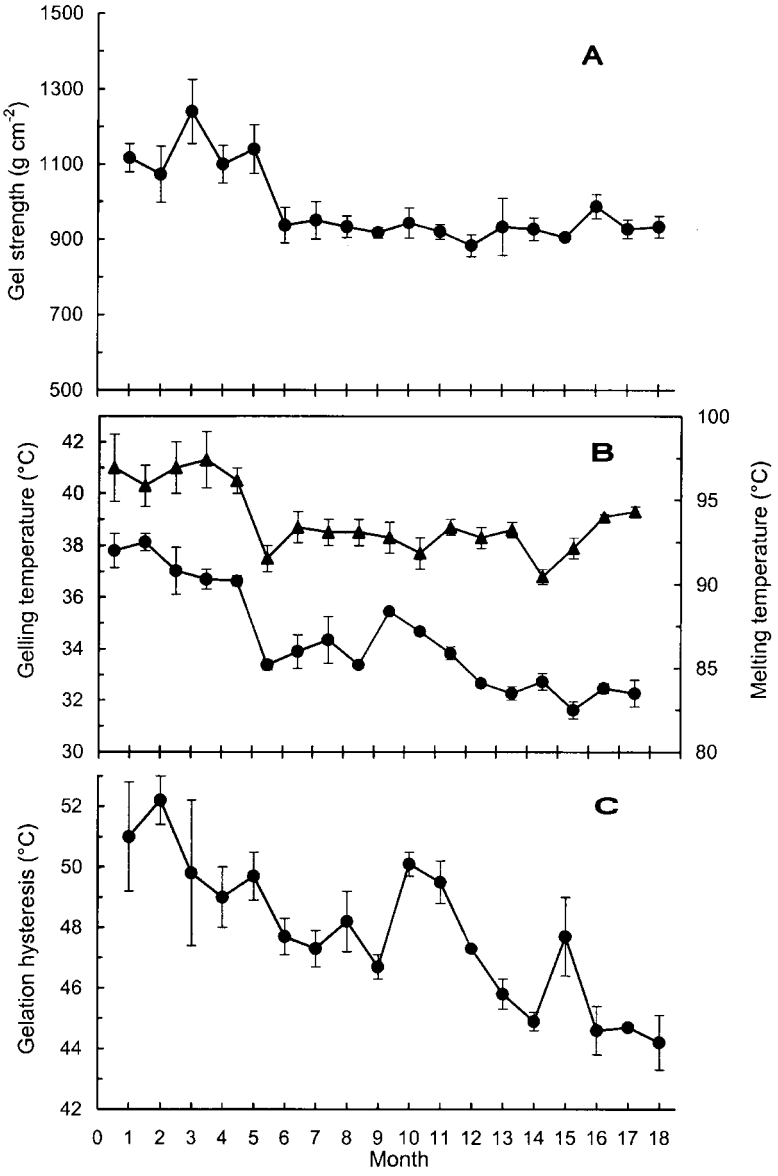

Figure 2. Physical properties along the study period. A. Gel strength. B. Gelling (●) and melting (▲) temperatures. C. Gelation hysteresis. Bars represent the standard deviation.

< 20% can be stored for nearly 10 years without loss of yield (Armisén, 1995). The same author points out that *G. sesquipedale* (Clem.) Thuret agar is extracted easily after one or two years, improving its yield.

The process responsible for agar degradation under unfavorable conditions could be of bacteriological or enzymatic origin (Armisén, 1995; Kapraun, 1999). Although we can not discriminate between this two processes, no changes in gel strength, gelling and melting temperature were observed until the sixth month of storage. Nevertheless, these changes accounted only for a 17% loss in gel strength and ~ 7% in gelling and melting temperatures. Although these parameters decreased, the values obtained until the end of the study kept quality characteristics within limits for other species of *Gracilaria* currently exploited in the world (Zemke-White & Ohno, 1999) as shown in Table 1. For instance, gel strength values are comparable, and even higher, to those of the most important species for agar production such as *G.*

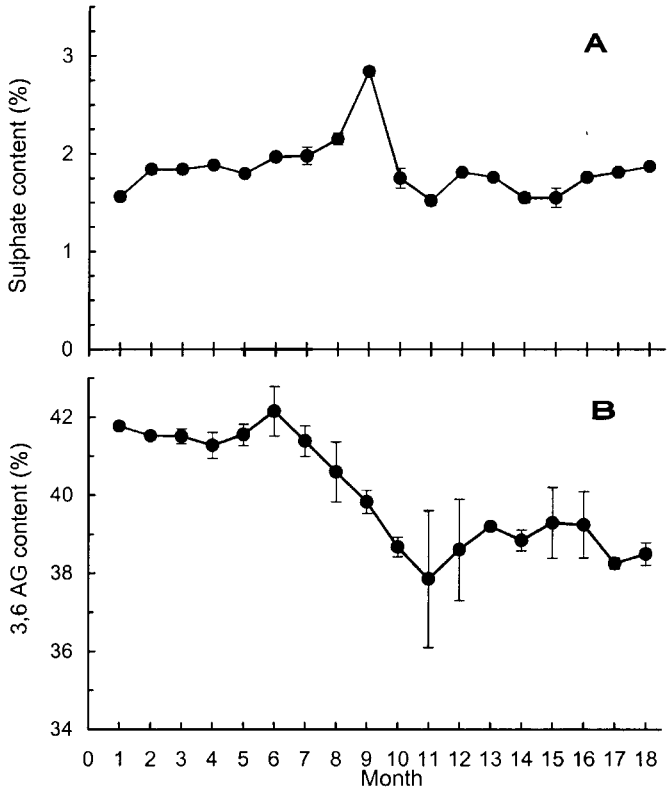

Figure 3. Chemical properties along the study period. A. Sulphate content. B. 3,6 AG content. Bars represent the standard deviation.

Table 1. Agar yield and gel strength from the most important *Gracilaria* species of the world

Species	Source	Yield (%)	Gel strength (g cm^{-2})	Reference
G. asiatica Zhang et Xia	China	24.1	620	Lian, 1996
G. chilensis Bird, McLachlan et Oliveira	Chile	43.4	360	Matsuhiro & Urzúa, 1990
G. edulis (Gmelin) Silva	India	43.0	120	Kalimuthu & Ramalingam, 1996
G. gracilis (Stackhouse) Steentoft	Southern Africa	17.1	859	Rebello et al., 1996
G. heteroclada (Motagne) Feldman et Feldman	Philippines	20.0	892	De la Peña, 1996
G. lemaneiformis (Bory) Dawson	Mexico	14.0	891	Pacheco-Ruíz et al., 1999
G. tenustipitata Chang et Xia	China	29.7	551	Lian, 1996
G. tenustipitata Chang et Xia	Philippines	16.2	726	De la Peña, 1996
G. cornea J Agardh	Mexico	19.2[a]	1134[a]	Present study
G. cornea J Agardh	Mexico	20.4[b]	930[b]	Present study

[a] Average agar yield and gel strength for the first five months of the study.
[b] Average agar yield and gel strength for the last thirteen months of the study.

chilensis Bird, McLachlan et Oliveira (Matsuhiro & Urzúa, 1990) and *G. gracilis* (Stackhouse) Steentoft (Rebello et al., 1996). Changes in gel strength after the fifth month are related to a decrease in 3,6 AG rather than to sulphate content variation. In fact, sulphate content showed no clear trend and had values below those required by the industry for this genus (< 4%). Armisén (1995) stated that cold water species, such as *G. chilensis*, resist better to hydrolysis during storage than warm water species and this characteristic is detected after 6–8 months, becoming important after one year. However, this author did not precise the storage conditions. The present report reveals that the warm water species *G. cornea* growing in Yucatán resists as

well as the Chilean species to degradation during storage conditions that are expected to resemble those of agar processing plants in temperate areas. An increase in temperature and humidity of storage could probably cause or accelerate agar degradation processes.

Previous results show that the gel strength of *G. cornea* agar from Yucatán depends on the season of the year, with the maximum during the dry season (1650 g cm^{-2}) and minimum in the cold season (635 g cm^{-2}) (Freile-Pelegrín & Robledo, 1997a). Although agar quality does not suffer a drastic degradation after one and a half years of storage, it is evident from these results that seasonality effects should be taken into consideration when harvesting biomass for agar production. Furthermore, choosing the harvest season to have environmental conditions for proper drying is also important. In this case, the optimum season to harvest and to dry seaweed coincide with the best agar quality from this specie.

It can be concluded that agar from *G. cornea* from Yucatán will suffer a very slow hydrolysis when the biomass is stored under conditions described in this study. This tropical *Gracilaria* can be stored for a reasonable time maintaining its agar quality, suitable for food purposes and interesting from an industrial point of view.

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