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Seasonal variation of agar from *Gracilaria vermiculophylla*, effect of alkali treatment time, and stability of its Colagar

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Abstract *Gracilaria vermiculophylla*, from Baja California Sur, Mexico, was studied in order to determine the seasonal variation of yield and quality of native and alkaline agar during 2007–2008. The highest alkaline agar yield was obtained in summer (17%) and the highest gel strength in spring (1,132 g cm^{-2}). The highest melting temperature was 98°C (winter). The highest gelling temperature was 68°C (summer). The values obtained are within the range of the most important *Gracilaria* species harvested worldwide. During the agar extraction step, the best results were obtained after 30 min of alkali treatment with sodium hydroxide (7%), after which the quality decreased significantly. We produced Colagar from *G. vermiculophylla* which consists of the seaweeds treated with sodium hydroxide and dried. The yield and quality of the agar obtained from the Colagar shows stability in both yield and quality during 1 year of storage, suggesting that alkali treatment is a good method of avoiding agar hydrolysis during storage.

Keywords *Gracilaria* · Agar · Seasonal variation · Gel strength · Storage · Baja California Sur

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Introduction

Gracilaria is the most important genus in the agar industry worldwide because of its availability in natural populations and culture possibilities, providing around 60% of the raw material for agar production (Freile-Pelegrín and Robledo 1997a; Guzmán-Urióstegui and Robledo 1999). Also, it can be used directly in regional dishes and salads, in agriculture as fertilizer, and as source of metabolites with therapeutic applications (Brock and Shintaku 1996; Iknur and Cirik 2004; McHugh 2003). Agar is located in the extra cellular matrix and is secreted by the Golgi apparatus; the extra cellular matrix is composed of two main elements, one fibrillar (cellulose) and other mucilaginous (agar; Armisén 1999). Agar is a polysaccharide (galactan). The idealized model for agar structure is represented for the agarobiose composed of two alternant monosaccharides of D-galactose and 3,6-anhydro-L-galactose (3,6-AG), which can be substituted in some degree by sulfate, methyl, or pyruvate groups depending on the extraction method (Armisén 1995; Armisén and Galatas 1987; Freile-Pelegrín and Robledo 1997a). Generally, sampling season significantly affects the yield and quality of agar. However, not all species present the same variation since the changes in chemical composition may be due to physiological factors or reproductive status of the population (Givernaud et al. 1999; Marinho-Soriano and Bourret 2005). The variation in agar quality for *G. vermiculophylla* is unknown. Large content of sulfate groups affect the agar quality; one way to solve it and the low content of agarose in *Gracilaria* agar is to use an alkaline treatment with sodium hydroxide (Armisén 1995; Pereira-Pachecho et al. 2007). Of all the process steps for agar extraction from *Gracilaria*, storage is the most difficult to solve because an enzymatic hydrolysis may occur, even with low moisture content. It varies depending on the

species and geographical origin. In *Gracilaria* species from the tropics, agar content decreases in a few months, but not in species from temperate and cold water, like *G. vermiculophylla*, because they are more resistant to hydrolysis during longer storage periods (Armisén and Galatas 1987). Nevertheless, the hydrolysis in *Gracilaria* species from cold waters is detected after 6 to 8 months of storage and becomes important after 1 year (Armisén 1995; Freile-Pelegrín 2000). The agar hydrolysis in *Gracilaria* could be due to two factors: the presence of agarolytic bacteria, of which the most important is *Bacillus cereus*, and the presence of the algae's own agarolytic enzyme (Armisén 1995).

Processed *Eucheuma* Seaweed or PES is a product with hydrocolloid properties obtained from either *Eucheuma cottonii* or *Eucheuma spinosum*. In addition to carrageenan polysaccharides, processed *Eucheuma* seaweed may contain 15% of insoluble algal cellulose and minor amounts of other insoluble matter. It is distinguished from carrageenan by its higher content of cellulosic matter, and it is not solubilized and precipitated during processing (Doty et al. 1987). There is a similar product obtained from red algae named Colagar. Therefore, a similar product is proposed in this study as an alternative for pre-processing the algae and avoiding storage degradation. Colagar may help the storage of pretreated seaweeds with no degradation. This is especially important for isolated fishing communities separate from large cities by difficult roads, like San Ignacio, BCS.

The objective of this research was to determine the seasonal variation of yield and physical properties of native and alkaline agar from *G. vermiculophylla*, determine the effect of alkaline treatment time at pilot plant level, to produce Colagar from *G. vermiculophylla*, and to determine its stability during storage.

Material and methods

Plants were collected in Laguna San Ignacio, Baja California Sur, Mexico (26°45′10.6″ N, 113°16′01.25″ W and 26°35′ 50.7″ N, 113°03′50.2″ W) by SCUBA diving at 2-m depth from autumn 2007 to summer 2008. The seasonal variation of agar yield and quality was determined in the laboratory. For the experiments at the pilot plant level, we used samples collected in 2004 and 2005. All were collected by hand and sun-dried, transported to the laboratory, and milled. Agar extractions (native and alkaline) were carried out in triplicate.

Native agar extraction Twenty-five grams of *G. vermiculophylla* was placed in 800 mL distilled water and heated until 80°C was reached, and the pH was adjusted to 6.2. Subsequently, it was heated until boiling for 90 min. After this time, the solution was mixed with diatomaceous earth and filtered by vacuum. The agar solution was allowed to gel at room temperature, frozen for 24 h, and thawed. The agar was dehydrated with ethanol and dried in an oven for 24 h at 55°C. After cooling, the agar was weighed to calculate the yield (Arvizu-Higuera et al. 2008).

Alkaline agar extraction Twenty-five grams of *G. vermiculophylla* was soaked 12 h in 500 mL NaOH (7%) at room temperature (23°C) to rehydrate the seaweed. The solution was heated at 85°C for 30 min with constant agitation. After alkali treatment, the samples were washed three times with 500 mL distilled water for 5 min and then treated with 500 mL H_2SO_4 (0.025%) for 2 h with constant agitation. To remove the excess of acid, the samples were washed with 500 mL distilled water for 10 min. Then, the samples were placed in 800 mL water, the pH was adjusted to 6.2 with phosphoric acid (10%), and the extraction process was performed as described above for native agar (Arvizu-Higuera et al. 2008).

Effect of alkaline treatment time at pilot plant level Ten kilogram of *G. vermiculophylla* was soaked 12 h in 200 L of NaOH (7%) in an extraction kettle at room temperature to hydrate the seaweeds. This was followed by 40 min of heating at 85°C. During the process, six samples of 150 g (wet) were obtained from the kettle at different times: before heating, after reaching 85°C, and then every 10 min until 40 min. In the laboratory, the samples were washed three times with 500 mL distilled water for 5 min and then treated with 500 mL H_2SO_4 (0.025%) for 2 h with constant agitation. To remove the excess of acid, samples were washed with 500 mL water for 10 min. Each sample was placed in 800 mL water. Then, the pH was adjusted to 6.2 and the extraction process was performed as described for native agar.

Production of Colagar from Gracilaria and its stability during storage This product was obtained in duplicate using algae with 2 and 3 years of storage. Ten kilograms of *G. vermiculophylla* was soaked 12 h in 200 L NaOH (7%) in an extraction kettle at room temperature to hydrate the seaweeds. This was followed by 40 min of heating at 85°C. The seaweeds were washed three times with 200 L water for 5 min, then were sieved to drain the water, sun-dried, milled, and stored at room temperature. Monthly samples (16 g) of Colagar were sampled during 1 year, after 1, 2, 3, 5, 7, 9, and 12 months of storage. Colagar samples were washed with 500 mL 0.025% H_2SO_4 for 2 h with constant agitation, and then the agar extraction was continued in the same way as described previously for native agar. The yield and

physical properties of the agar obtained from the Colagar were evaluated in triplicate.

Physical properties An agar solution (1.5%) was prepared to measure physical properties. Agar gels were prepared in plastic containers (3×9×2.5 cm) filled with agar solution and allowed to gel at room temperature (22°C). Gel strength was measured using a modified Nikan-Sui gelometer (Armisén and Galatas 1987). Melting temperature was measured in test tubes (1.7-cm diameter, 15-cm height), placing an iron ball (7-mm diameter) on the surface of the gel. The test tube was heated in a water bath with constant agitation, and the temperature increased gradually until boiling. Melting temperature was recorded with a precision thermocouple thermometer when the gel started to melt and the ball sank into the solution. Gelling temperature was measured using the same melted solution described above. The solution was allowed to cool down while moving the tube from vertical to almost horizontal. When the solution ceased flowing, temperature was recorded with a thermocouple (Arvizu-Higuera et al. 2008). Average and standard deviation (±1 SD) were computed. An ANOVA or Kruskall–Wallis test (depending if the data were normal or not) were used to detect significant differences among treatments ($p<0.05$).

Results

Seasonal variation of the yield and physical properties of native and alkaline agar of *G. vermiculophylla*

Significantly higher agar yield was obtained in summer for native agar (29%) and alkaline agar (17%). Also, significantly higher yield ($p<0.05$) was obtained for native agar than alkaline agar in all seasons, with a maximum difference of 52% in spring (Fig. 1a). The gel strength for native and alkaline agar showed significant seasonal variations ($p<0.05$). The alkaline-treated agar was significantly stronger ($p<0.05$, Fig. 1b) than native agar. Highest values were obtained in summer for native agar (170 g cm^{-2}) and spring for alkaline agar (1,132 gcm^{-2}). The average gel strength for alkaline agar was nine times higher than native agar ($p<0.05$). The melting temperature (Fig. 1c) of native agar had significant seasonal variations ($p<0.05$) with a gradual increase from autumn (66°C) to summer (75°C), while no significant seasonal variation was obtained for alkaline agar (average 94°C). The average melting temperature of native agar increased significantly (32%) after alkaline treatment. Gelling temperature (Fig. 1d) of native agar varied significantly during the year, with the minimum in autumn (24°C) and the maximum in

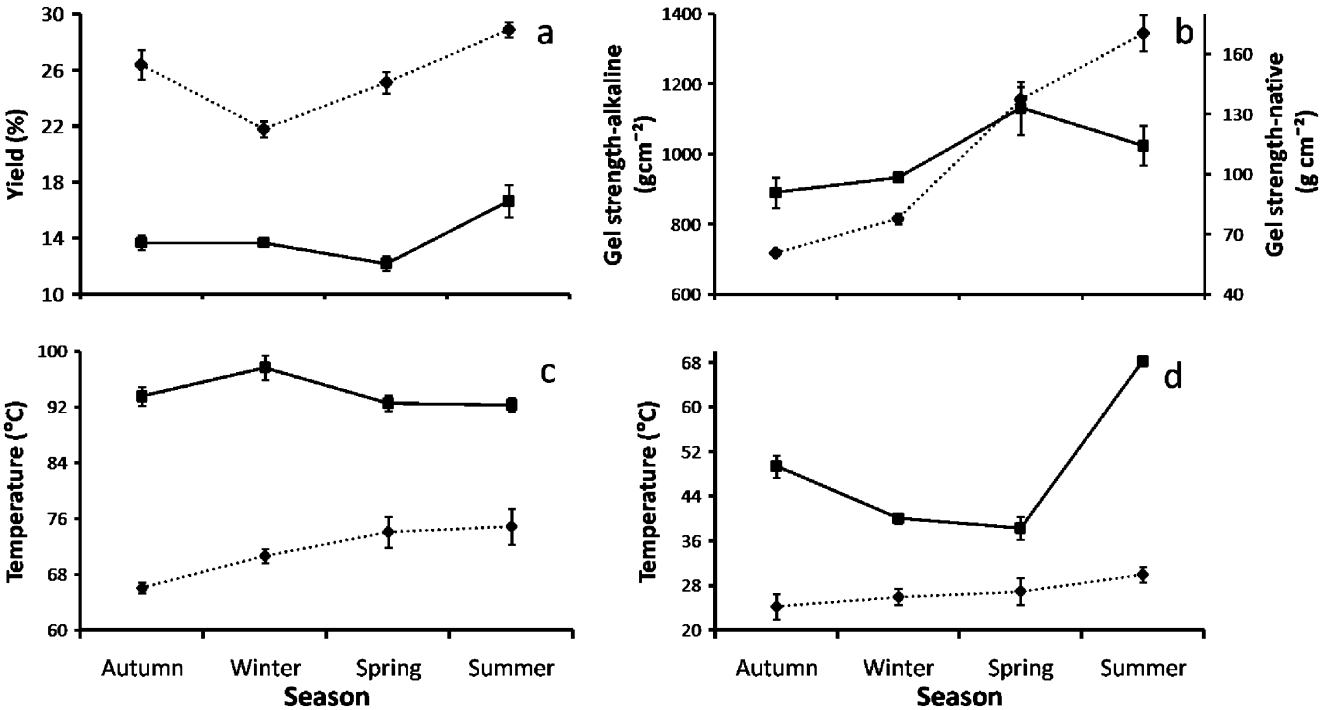

Fig. 1 Seasonal variation of the physical properties and yield of *G. vermiculophylla* agar: native (*dashed line*) and alkali-treated (*solid line*). **a** Agar yield. **b** Gel strength. **c** Melting temperature. **d** Gelling temperature. Data=mean±SD

summer (30°C). The alkaline agar varied significantly, with maximum values in summer (68°C). The values were significantly increased after alkaline treatment (average 81.5%)

Effect of alkali treatment time at the pilot plant level

The alkali treatment produced a significant reduction of agar yield (40% lower) for the algae stored for 3 years, but no significant reduction for the algae stored for 2 years (Fig. 2a). The gel strength for the algae stored for 2 years was five times higher than the gel strength of the algae stored for 3 years. Significant differences ($p<0.05$) were observed among the samples from the algae 3 years old, while for the algae stored for 2 years, there were no significant differences ($p>0.05$) in spite of the increase nearly to 100 $g cm^{-2}$ (Fig. 2b). The melting temperature had a significant increase in both algae with two and three storage years (Fig. 2c). The greatest effect of alkali treatment on the melting point was observed in the algae stored for 3 years, with an increase of nearly 30%. The increase in melting point for the algae stored for 2 years was 10%. The algae stored for 2 years had a higher melting temperature than algae stored for 3 years in all sampled times (Fig. 2c). The gelling temperature (Fig. 2d) showed the same trend as the melting temperature. The algae stored for 2 and 3 years showed a significant increase in the gelling point ($p<0.05$), and the algae stored for 2 years had the highest values of gelling temperature at all times.

Effect of storage time on Colagar from *G. vermiculophylla*

Significant differences were observed ($p<0.05$) among some months in all the physical properties (Fig. 3a–d). Significant differences were obtained between the algae stored for 2 years and algae stored for 3 years in yield and all the other properties. The algae stored for 2 years had higher values than the algae stored for 3 years for all of the parameters measured. The analysis along the storage time showed that the slope was not significantly different from zero. This means that the yield and physical properties remained constant at least during the 12 months analyzed.

Discussion

The average agar yield was higher after alkaline treatment than native agar (Fig. 1a); however, in both cases, the values obtained were similar or higher than values reported

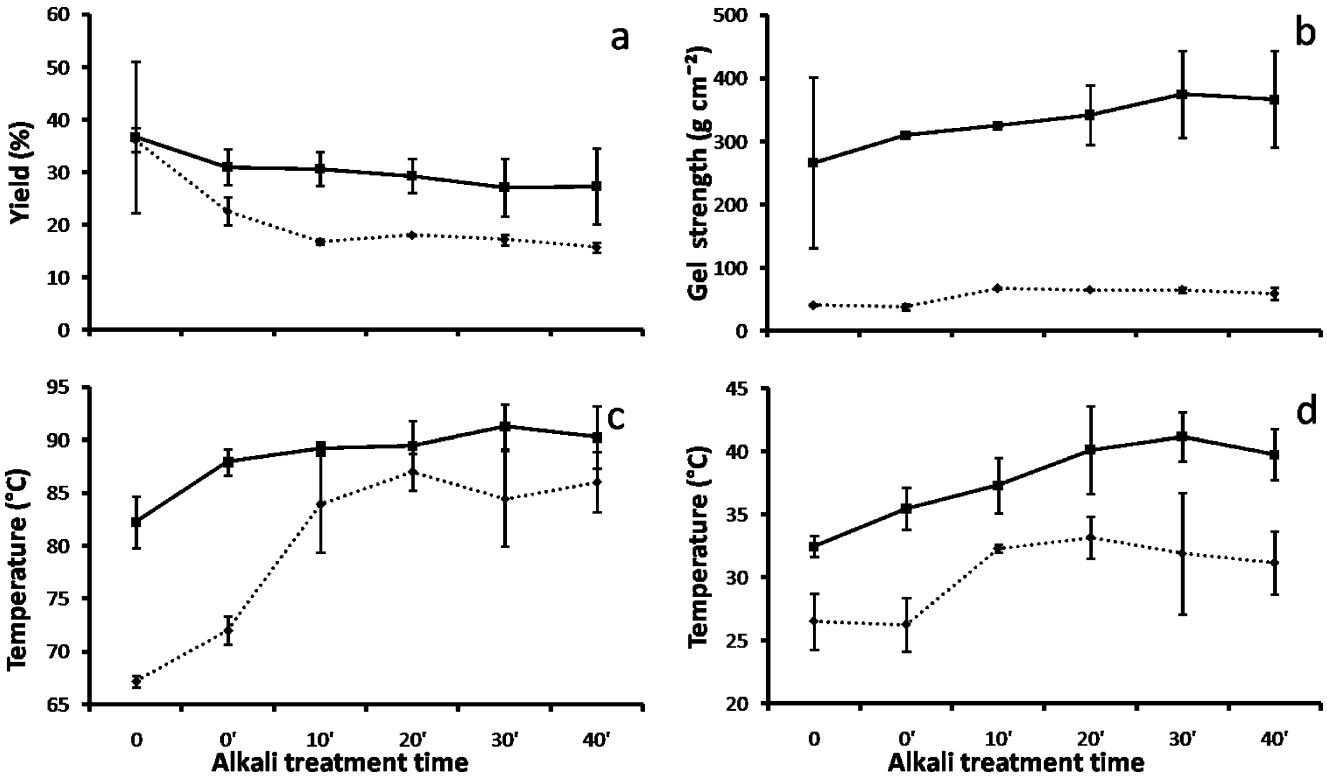

Fig. 2 Physical properties and yield of *G. vermiculophylla* agar during alkaline treatment: algae with 3 years of storage (*dashed line*) and 2 years (*solid line*). **a** Agar yield. **b** Gel strength. **c** Melting temperature. **d** Gelling temperature. Data=mean±SD

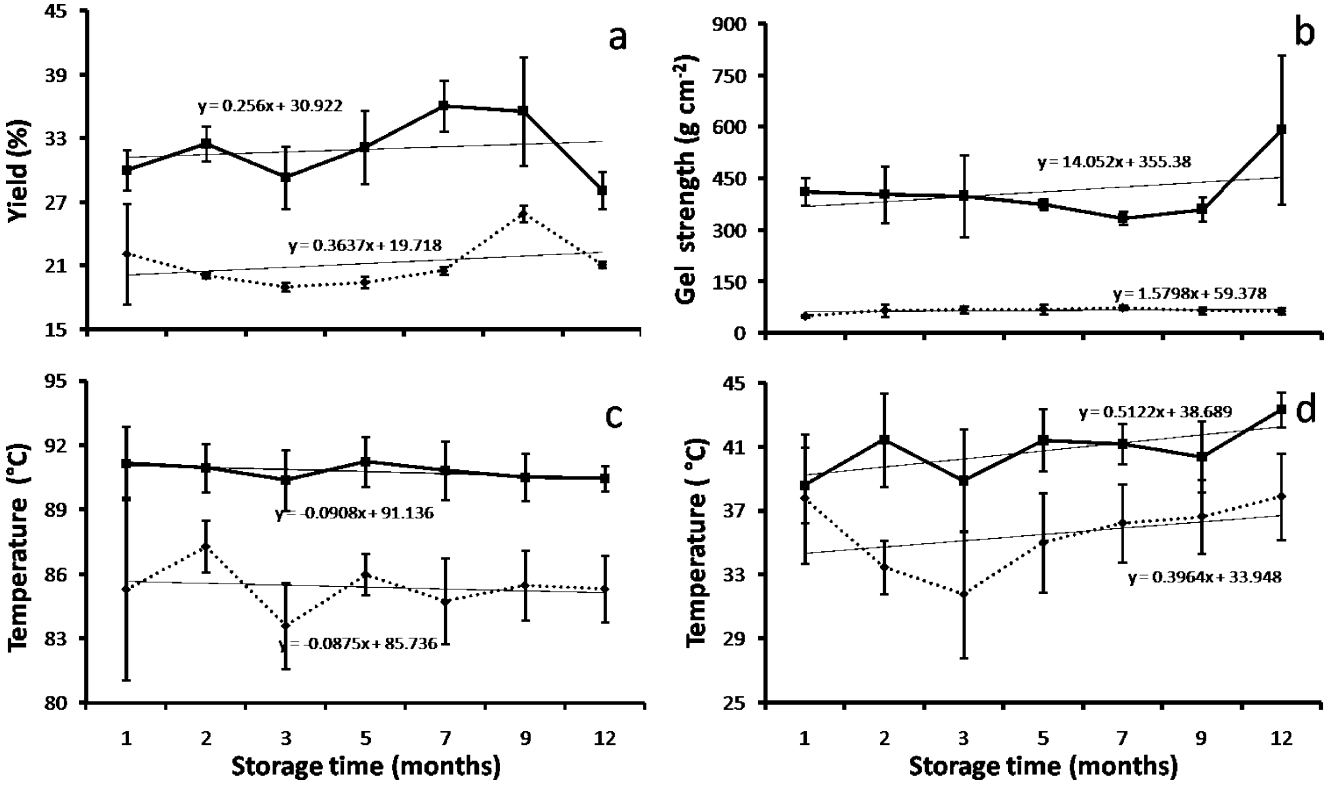

Fig. 3 Yield and physical properties of the agar obtained from the Colagar storage at different times: 3 years (*dashed line*) and 2 years (*solid line*). **a** Agar yield. **b** Gel strength. **c** Melting temperature. **d** Gelling temperature. Data=mean±SD

for the most important *Gracilaria* species used worldwide and other *G. vermiculophylla* populations (Table 1). The gel strength of the alkaline agar from spring and summer was higher than any other species showed in Table 1 (Fig. 1b). The maximum yield and quality were obtained when maximum biomass of *G. vermiculophylla* was present at Laguna San Ignacio (1,004 wet tons in spring) and when the population was mature and biomass was reducing (228 wet tons in summer; Vergara-Rodarte 2009). The differences in agar yield and quality found among seasons may be related to seasonal changes in environmental conditions (Ondarza 2007), the reproductive state of the population (Givernaud et al. 1999), and/or the increase of algae size along the year, which lead to the algae maturity in summer

Table 1 Yield and gel strength of the *Gracilaria* species more important worldwide and *G. vermiculophylla* analyzed in other studies (modified from Freile-Pelegrín 2000)

Species	Origin	Yield (%)	Gel strength (g cm^{-2})	Reference
G. asiatica	China	24.1	620	Lian (1996)
G. chilensis	Chile	43.4	360	Matsuhiro and Urzua (1990)
G. edulis	India	43.0	120	Kalimuthu and Ramalingan (1996)
G. gracilis	South Africa	17.1	859	Rebello et al. (1996)
G. heterocladia	Philippines	20.0	892	De la Peña (1996)
G. lemaneiformis	Mexico	14.0	891	Pacheco-Ruíz et al. (1999)
G. tenuistipitata	China	29.7	551	Lian (1996)
G. tenuistipitata	Philippines	16.2	726	De la Peña (1996)
G. vermiculophylla	Mexico	10.2	177	Orduña-Rojas et al. (2008)
G. vermiculophylla	France	17.8	195	Mollet et al. (1998)
G. vermiculophylla	Mexico	15.3	1,064	Arvizu-Higuera et al. (2008)
G. vermiculophylla	Mexico	17.0	1,132	This study (alkaline agar)
G. vermiculophylla	Mexico	28	170	This study (native agar)

(Vergara-Rodarte 2009). The seasonal variation of gel strength is particular for each species. For example, the highest gel strength for *G. gracilis* was in autumn–winter and for and *G. bursa-pastori* was in spring–summer even when the samples were collected in the same site and season (Marinho-Soriano and Bourret 2003). It is well documented that the increase in gel strength after alkaline treatment is related to the presence of an ester sulfate in the C-6 oxygen of the galactose unit linked in C-4, and these residues with this kind of substitution in the C-6 are precursors of 3,6-AG, and also that agars with high content of 3,6-AG produce strong gels and, vice versa, agars poor in 3,6-AG produce weak gels (Armisén 1995; Duckworth et al. 1971; Montaño et al. 1999).

The results obtained at pilot plant for the alkaline treatment showed differences between the 2 and 3 years of storage time in yield and quality. Both showed a yield reduction as the treatment time progressed, but it was significant only for the 3-year storage period (Fig. 2a). After 40 min of treatment, yield, gel strength, and gelling temperature reached the asymptotic part of the graph; because of that, 30 min was considered the best time of alkali treatment for *G. vermiculophylla*. Arvizu-Higuera et al. (2008) suggested the same time after experimenting longer time periods (0.5, 1, 1.5, 2, 2.5, and 3 h), concluding that longer times of alkali treatment reduced the 3,6-AG. The yield decrease along the treatment time could be attributed to the degradation and the loss of polysaccharides in the alkaline solution and the elimination of floridean starch during filtrations (Freile-Pelegrín and Robledo 1997b; Meena et al. 2008); therefore, an increase in the time and alkali concentration could have a negative effect on the agar yield.

The storage time is a critical matter for the agar industry because sometimes, it is impractical to process all the algae harvested. Therefore, the industry needs to have stored stock (Armisén and Galatas 1987). The most important factor after harvesting algae is correct drying and packing. The appropriate process is to dry the algae to <20% moisture content and avoid wetting during the transporting and storage period. High moisture content in the package creates favorable conditions for anaerobic fermentation (Armisén 1995; Armisén and Galatas 1987). When the alkali-pretreated *Gracilaria* (Colagar) was used to obtain agar after different storage times, a positive trend was observed in the yield during the study year, which could be a consequence of the partial maceration of the cellular wall during the storage, which makes the agar extraction easier (Armisén 1995; Freile-Pelegrín 2000). Similar effects were observed for *G. eucheumatoides* (Romero et al. 2008) and *G. cornea* (Freile-Pelegrín 2000). The statistical analysis of regression between time and yield and the other quality factors showed that the slope was not different from zero. It suggests that pretreated alga (Colagar) remains stable for at least 1 year. In another genus, *Gelidium sesquipedale,* the agar yield may increase after 1 or 2 years of storage without decreasing in quality (Armisén 1995). That is because of the great resistance of *Gelidium* during storage. Our results suggest that it is not the case for *Gracilaria* and more caution should be taken because of the possibility of an enzymatic hydrolysis even with adequate moisture content. The agar hydrolysis in *Gracilaria* could be caused by two factors: the presence of agarolytic bacteria, from which the most important is *B. cereus*, and the algae's own agarolytic enzymes (Armisén 1995). Storage of *G. cornea* for 2 years produced a reduction of 17% in gel strength and 12% in 3,6-AG (Freile-Pelegrín 2000). Also, in *G. eucheumatoides*, the reduction of gel strength after 1 year of storage was 35% and 7% in 3,6-AG (Romero et al. 2008). Different methods have been studied to prevent the enzymatic hydrolysis of algae. One of those is alkaline treatment which destroys the bacteria and denatures the agarolytic enzymes (Armisén 1995). Previously, in the 1960s, gamma rays were studied as a possible antibiotic method; nevertheless, it was expensive and the gel strength was lower than food grade agar (Doshi et al. 1968). Our results suggest that alkaline treatment is a good method to preserve the yield and agar quality during storage. Considering the results obtained, we suggest that *G. vermiculophylla* could be considered a species with potential for commercial extraction of food grade agar.

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References

Armisén R (1995) World-wide use and importance of *Gracilaria*. J Appl Phycol 7:231–243

Armisén R (1999) Agar. In: Imenson A (ed) Thickening and gelling agents for food, 2nd edn. Blackie Academic & Professional, England, pp 1–21

Armisén R, Galatas F (1987) Production, properties and uses of agar. In: D. J. McHugh (ed) Production and utilization of products from commercial seaweeds. FAO Fisheries Technical Paper, Rome, pp 1–44

Arvizu-Higuera DL, Rodríguez-Montesinos YE, Murillo-Álvarez JI, Muñoz-Ochoa M, Hernández-Carmona G (2008) Effect of alkali treatment time and extraction time on agar from *Gracilaria vermiculophylla*. J Appl Phycol 20:515–519

Brock J, Shintaku M (1996) *Gracilaria* gall syndrome. Center for Tropical and Subtropical Aquaculture 124:2

De la Peña PO (1996) Philippines part II. In: FAO/NACA (ed) Regional study and workshop on the taxonomy, ecology and processing of economically important red seaweed. Bangkok: NACA Environment and Aquaculture Development Series No. 3, pp 143–149

Doshi YA, Talreja ST, Rao PS (1968) Production of high quality agar by gamma irradiation of seaweeds. Indian J Technol 6:275

Doty MS, Caddy JF, Santelices B (1987) Case studies of seven commercial seaweed resources. FAO Fisheries Technical Paper, Rome

Duckworth M, Hong KC, Yaphe W (1971) The agar polysaccharides of *Gracilaria* species. Carbohydr Res 18:1–9

Freile-Pelegrín Y (2000) Does storage time influence yield and agar properties in the tropical agarophyte *Gracilaria cornea*? J Appl Phycol 12:153–158

Freile-Pelegrín Y, Robledo D (1997a) Effects of season on the agar content and chemical characteristics of *Gracilaria cornea* from Yucatan, Mexico. Bot Mar 40:285–290

Freile-Pelegrín Y, Robledo D (1997b) Influence of alkali treatment on agar from *Gracilaria cornea* from Yucatán, Mexico. J Appl Phycol 9:533–539

Givernaud T, El Gourji A, Mouradi-Givernaud A, Lemoine Y, Chiadmi N (1999) Seasonal variations of growth and agar composition of *Gracilaria multipartita* harvested along the Atlantic coast of Morocco. Hydrobiologia 398(399):167–172

Guzmán-Urióstegui A, Robledo D (1999) Factors affecting sporulation of *Gracilaria cornea* (Gracilariales, Rhodophyta) carposporophytes from Yucatan, Mexico. Hydrobiologia 398(399): 285–290

Iknur A, Cirik S (2004) Distribution of *Gracilaria verrucosa* (Hudson) Papenfuss (Rhodophyta) in Izmir Bay (eastern Aegean Sea). Pak J Biol Sci 7(11):2022–2023

Kalimuthu S, Ramalingam JR (1996) India. In: FAO/NACA (ed) Regional study and workshop on the taxonomy, ecology and processing of economically important red seaweed. Bangkok: NACA Environment and Aquaculture Development Series No. 3, pp 73–86

Lian P (1996) China. In: FAO/NACA (ed) Regional study and workshop on the taxonomy, ecology and processing of economically important red seaweed. Bangkok: NACA Environment and Aquaculture Development Series No. 3, pp 53–72

Marinho-Soriano E, Bourret E (2003) Effects of season on the yield and quality of agar from *Gracilaria* species (Gracilariaceae, Rhodophyta). Bioresour Technol 90(3):329–333

Marinho-Soriano E, Bourret E (2005) Polysaccharides from the red seaweed *Gracilaria dura* (Gracilariales, Rhodophyta). Bioresour Technol 96:379–382

Matsuhiro B, Urzúa C (1990) Agars from *Gracilaria chilensis* (Gracilariales). J Appl Phycol 2:273–279

McHugh DJ (2003) A guide to the seaweed industry. FAO Fisheries Technical Paper, Rome

Meena R, Prasad K, Ganesan M, Siddhanta AK (2008) Superior quality agar from *Gracilaria* species (Gracilariales, Rhodophyta) collected from the Gulf of Mannar, India. J Appl Phycol 20:397–402

Mollet J-C, Rahaoui A, Lemoine Y (1998) Yield, chemical composition and gel strength of agarocolloids of *Gracilaria gracilis, Gracilaria longissima* and the newly reported *Gracilaria* cf. *vermiculophylla* from Roscoff (Brittany, France). J Appl Phycol 10:59–66

Montaño NE, Villanueva RD, Romero JB (1999) Chemical characteristics and gelling properties of agar from tow Philippine *Gracilaria* spp. (Gracilariales, Rhodophyta). J Appl Phycol 11:27–34

Ondarza MA (2007) Substituciones en la unidad D-Galactosa de polímeros del agar: implicaciones metabólicas. Revista de Biología Marina y Oceanografía 42(2):201–204

Orduña-Rojas J, Suárez-Castro R, López-Álvarez ES, Riosmena-Rodríguez R, Pacheco-Ruiz I, Zertuche-González JA, Meling-López AE et al (2008) Influence of alkali treatment on agar from *Gracilariopsis longissima* and *Gracilaria vermiculophylla* from the Gulf of California, Mexico. Ciencias Marinas 34(4):503–511

Pacheco-Ruíz I, Zertuche-González J, Correa-Díaz F (1999) *Gracilariopsis lemaneiformis* beds along the west coast of the Gulf of California, Mexico. Hydrobiologia 398/399:509–514

Pereira-Pachecho F, Robledo D, Rodríguez-Carvajal L, Freile-Pelegrín Y (2007) Optimization of native agar extraction from *Hydropuntia cornea* from Yucatan, Mexico. Bioresour Technol 98:1278–1284

Rebello J, Ohno M, Critchley AT, Sawamura M (1996) Growth rates and agar quality of *Gracilaria gracilis* (Stackhouse) Steentoft from Namibia, Southern Africa. Bot Mar 39:273–279

Romero JB, Villanueva RD, Montaño MNE (2008) Stability of agar in the seaweed *Gracilaria eucheumatoides* (Gracilariales, Rhodophyta) during postharvest storage. Bioresour Technol 99:8151–8155

Vergara-Rodarte MA (2009). Evaluación de biomasa y extracción de agar del alga roja *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta) de Laguna San Ignacio, BCS, México. Master's thesis, p 65