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Effect of alkali treatment time and extraction time on agar from *Gracilaria vermiculophylla*

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Abstract The effects of alkali treatment time and extraction time of native agar and alkali treated agar obtained from *Gracilaria vermiculophylla* were studied. The response characteristics were mainly agar yield and gel strength. Alkali treatment was carried out at 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h. Agar yield and gel strength decreased with the increase in the time of the alkali treatment. The highest yield (15.3%) and highest gel strength (1,064 g cm ^{2}) were obtained at 0.5 h, and therefore this time was used for the next experiment. The extraction of both native and alkali treated agars was carried out at 1.5, 2.0, 2.5, and 3.0 h. The best extraction time for alkali treated agar was 1.5 h, and for native agar 2.5 h. The alkali treated agar obtained with the different alkali treatment and extraction times showed higher melting (92.4–99.7°C) and gelling (35.7–39.6°C) temperatures. Native agar was lower in melting (60.2–64.1°C) and gelling (20.4–23.4°C) temperatures. The 3,6-anhydrogalactose content decreased with increasing alkali treatment time, with the opposite effect during the extraction of native and alkali treated agars.

Keywords Agar yield · 3,6-anhydrogalactose · Gel strength · Melting and gelling temperatures · Polysaccharide

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Introduction

Agar is a polysaccharide comprised of two major components, agarose and agaropectin. Agarose is a neutral polysaccharide with a linear structure of repeating units of the disaccharide agarobiose, a dimmer of D-galactose and 3,6-anhydro-L-galactose. Agaropectin is an acid polysaccharide containing sulfate groups, pyruvic acid, and D-glucuronic acid conjugated to agarobiose (Araki 1966; Duckworth and Yaphe 1971; Yaphe 1984). Agar is obtained from some families of red algae (Rhodophyceae), mainly Gelidiaceae and Gracilariaceae. Agar is used as a food ingredient, accounting for 80% of its consumption. The remaining 20% is used for biotechnological applications. Agar was the first phycocolloid used in the human food industry (Armisen and Galatas 2000).

Agar is obtained by leaching of the alga in hot water, filtering off the extract, and separating the agar by freezing and thawing to eliminate the water (Armisen and Galatas 1987). The yield and quality of agar varies with the species and environmental factors related to the alga growth, light, nutrients, and temperature (Santelices 1988). Although the general aspects of the extraction process are known, studies are still needed to increase the yield and quality of the product. Agar of some algae, such as *Gelidium*, is easily extracted with boiling water. In other species, like *Gracilaria*, a pretreatment with alkali (NaOH) is needed for the desulphation of the native agar, causing the formation of a 3,6-anhydrogalactose bridge and an increase in the gel strength of the alkali treated agar (Freile-Pelegrín and Robledo 1997). The type of treatment before the extraction and the extraction time both affect the yield and quality of the agar.

The harvesting of red seaweeds started in Mexico in the 1940s. In 1960, AGARMEX set up an small agar extraction

plant in Ensenada, Baja California. From 1970, the production was increased to process 1,200 t of dried seaweeds, and it remains the same at the present time. The raw material is *Gelidium robustum* (N.L. Gardner) Hollenberg & I.A. Abbott, which is harvested from Punta Descanso, Baja California, to Punta San Hipólito, Baja California Sur (Zertuche-González 1993). *Gelidium robustum* is the only species of red algae exploited commercially. However, Zertuche-González (1993) pointed out that extensive beds (60 ha) of another red seaweed, *Gracilaria vermiculophylla* (Ohmi) Pappenfus (former *pacifica*), were found in Laguna San Ignacio, Baja California Sur, with an average biomass ranging from 5 to 15 kg m^{-2} (wet weight). He estimated that 900 t per year (dry weight) could be harvested in that area. In 1993, 20 t of dried *G. vermiculophylla* were exported to China, but at present no one company is harvesting the beds. Recent studies confirmed the abundance of this species (Vergara-Rodarte 2006), but the characteristics of the agar remains unknown. The above data suggest that beds with economic potencial exist in Baja California, but the best extraction methods and chemical characteristic of its agar have not been studied in order to decide about industrial exploitation. Our objective was to study the effect of alkali treatment and extraction times on the yield and some physical and chemical properties of the resulting agar.

Material and methods

Gracilaria vermiculophylla was collected in Laguna San Ignacio, BCS, Mexico (26°45′10.6″N, 113°16′01.25″W and 26°35′50.7″N, 113°03′50.2″W) by diving at 3–5 m depth in April 2005. The plants were washed with 3% formaldehyde to prevent enzymatic hydrolysis, sun dried, and milled to 20-mesh size.

Effect of alkali treatment time

The alkaline treatment and the agar extraction were done according to the method described by Freile-Pelegrín and Robledo (1997), with some modifications. Twenty-five grams of *Gracilaria vermiculophylla* were soaked 12 h in 500 ml of 7% NaOH at room temperature (22°C) to rehydrate the seaweed. Then the solution was heated in a water bath at 85°C for 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h, with constant agitation.

Agar extraction

After alkali treatment, each sample was washed three times with 500 mL of distilled water for 5 min, and then washed with 500 mL of 0.025% H_2SO_4 for 2 h with constant agitation. To remove excess acid, samples were washed with 500 mL of distilled water for 10 min. The algae were placed in 900 mL of hot distilled water with constant agitation. When the solution reached 80°C, the pH was adjusted to 6.2–6.5 with 10% phosphoric acid. The algae were boiled for 1.5 h. The extract was mixed with diatomaceous earth (Celite) and filtered using vacuum. The filtrate was allowed to gel at room temperature on plastic trays, frozen overnight, and thawed at room temperature. Finally, the agar was washed with ethanol, dried in an oven at 55°C for 24 h, cooled, and weighed to calculate the percentage yield of agar.

Effect of extraction time on alkali treated agar

Twenty-five grams of algae were soaked overnight in 500 mL of 7% NaOH at room temperature. The solution was heated in a water bath at 85°C for 0.5 h (this time was selected using the results obtained in the above experiment). Agar extraction was done as described above. The algae were boiled for four different times, 1.5, 2.0, 2.5, and 3.0 h.

Effect of extraction time on native agar

Twenty-five grams of algae were placed in 900 mL of hot distilled water. When the solution reached 80°C, the pH was adjusted to 6.2–6.5 with 10% phosphoric acid. The algae were boiled for four different times, 1.5, 2.0, 2.5, and 3.0 h. The extract was mixed with diatomaceous earth and filtered by using vacuum. The filtrate was allowed to gel at room temperature, frozen overnight, and then thawed. Finally, the agar was washed with 90% ethanol, dried in an oven at 55°C for 24 h, cooled, and weighed to calculate the percentage yield of agar.

Physical properties

A 1.5% agar solution (w/v) was prepared to measure the physical properties: gel strength, melting point, and gelling temperature. Agar solutions were allowed to gel at room temperature (22°C) overnight in plastic containers (3×9×2.5 cm). Gel strength was measured using a modified Nikansui Shiki gelometer. The melting temperature was measured by preparing the agar gel in a test tube (1.7 cm diameter, 15 cm height) and placing an iron ball (7 mm diameter) on the surface of the gel. The test tube was clamped in a water-bath and the temperature increased gradually. The melting temperature was recorded with a precision thermocouple thermometer when the gel started to melt and the ball sank into the solution.

The gelling temperature was measured using the same hot solution in the tube above. The solution was allowed to cool down while moving the tube continuously, vertical to almost horizontal. The probe of the thermocouple was

introduced in the solution when it ceased flowing, and temperature was recorded. Hysteresis was calculated as the difference between melting and gelling temperature.

Chemical properties

The percentage of 3,6-anhydrogalactose (3,6-AG) was determined by the resorcinol-acetal method (Yaphe and Arsenault 1965) using D-fructose as the standard. Absorbance was read at 555 nm.

Statistical analysis

All extractions and agar analysis were made in triplicate. Data were computed to obtain the average and standard error (±1 SE) with a significance level at 95%. An ANOVA was used to detect significant differences among treatments ($P<0.05$).

Results

Effect of alkali treatment time

Significantly higher yield (15.3%) and gel strength (1, 064 g cm^{-2}) were obtained at 0.5 h of alkali treatment, these values decreasing as the treatment time increased (Fig. 1). The melting temperature (94.2°C), gelling temperature (36.6°C) and hysteresis (57.6°C) were lower after 0.5 h of treatment. The maximum values were obtained at 3 h of alkali treatment for melting temperature (99.7°C) and hysteresis (61.4°C). The maximum gelling temperature (39.6°C) was at 1 h of treatment. No significant differences were found among the values obtained after 1 h. The highest value for 3,6-AG was at 1 h (48.9%), decreasing to a minimum (38.9%) at 3 h of alkali treatment (Table 1).

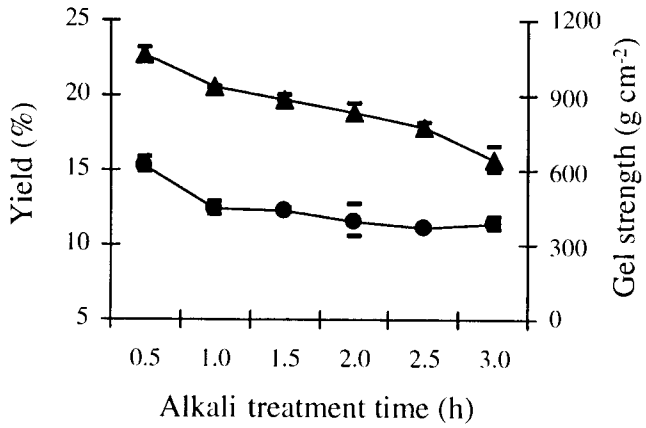

Fig. 1 Agar yield (●) and gel strength of 1.5% agar (▲) from *Gracilaria vermiculophylla* with different alkali treatment times. *Bars* represent standard error (±SE)

Effect of extraction time on alkali treated agar

Figure 2a shows an inverse relation between agar yield and gel strength, with the minimum yield (15.4%) and maximum gel strength (1,064 g cm^{-2}) obtained at 1.5 h of extraction. Significant differences were found between all treatment times for both characteristics. Higher values of melting temperature (98.1°C), gelling temperature (37.8°C), and hysteresis (60.3°C) were obtained after extraction for 2 h and the lower values were obtained at 3 h with 92.4°C, 35.7°C, and 56.7°C, respectively (Table 1). The 3,6-AG content showed a direct relationship with the extraction time, obtaining the minimum value of 42.7% at 1.5 h and the maximum of 45.7% at 3 h extraction time (Table 1).

Effect of extraction time on native agar

The yield of native agar was significantly higher than that of alkali treated agar. The agar yields for all treatments ranged from 29.7–34.6% and were significantly different at different extraction times. The highest yield was obtained at 1.5 h of extraction and the lowest at 2.5 h (Fig. 2b). Variation in gel strength was also significantly different among extraction times, and significantly lower than for alkali treated agar (Fig. 2b). The maximum value was 72 g cm^{-2} at 2.5 h of extraction and the minimum was 46 g cm^{-2} at 1.5 h. Melting temperature, gelling temperature, and hysteresis were significantly different among treatments. Higher values of melting (64.1°C) and gelling temperature (23.4°C) were obtained at 2.5 h, though maximum hysteresis (43.3°C) was obtained at 1.5 h (Table 1). These three characteristics had significantly lower values than those for alkali treated agar. The 3,6-AG content showed a similar trend to that for the extraction time in the alkali treated agar experiment, but significantly lower, from 30.5% at 1.5 h to 37.3% at 3 h (Table 1).

Discussion

From our results, the alkali treatment time had a significant effect on the agar yield. The yield was reduced with treatment time. However, we observed that during the washes of the product there was little agar loss by diffusion. The agar yield was lower than reported by Hurtado-Ponce and Umezaki (1988) for another species of *Gracilaria* from the Philippines, reported to be 23–48%.

Alkali treated agar from *G. vermiculophylla* had a gel strength from 644 to 1,064 g cm^{-2} similar to commercial agar (>800 g cm^{-2}). Mollet et al. (1998) reported lower values in the same species without gel strength improvement after alkali treatment. This suggests that yield and gel strength from some species are mainly dependent on the

Table 1 Physical properties and 3,6-anhydrogalactose content of agar from *Gracilaria vermiculophylla* with different treatments

Treatment	Time (h)	Melting temperature (°C)	Gelling temperature (°C)	Hysteresis temperature (°C)	3,6-AG (%)
Effect of alkali treatment time	0.5	94.2±0.7	36.6±1.1	57.6±1.3	42.7±0.6
	1.0	99.5±0.3	39.6±0.3	59.8±0.5	48.9±1.2
	1.5	98.3±0.6	39.1±0.7	59.1±0.6	46.1±2.6
	2.0	99.0±0.7	38.1±0.8	61.0±1.1	44.1±1.7
	2.5	99.4±0.3	39.6±0.3	59.8±0.5	45.5±0.5
	3.0	99.7±0.3	38.3±0.6	61.4±0.4	38.9±0.7
Effect of extraction time on alkali treated agar	1.5	94.5±0.4	36.5±1.3	58.0±1.2	42.7±0.6
	2.0	98.1±1.1	37.8±0.6	60.3±1.7	44.4±0.7
	2.5	95.6±1.2	35.7±0.4	59.9±1.2	44.8±1.6
	3.0	92.4±1.2	35.7±0.8	56.7±0.9	45.7±2.0
Effect of extraction time on native agar	1.5	63.7±0.5	20.4±0.3	43.3±0.6	30.5±0.3
	2.0	60.2±0.5	22.3±0.4	37.9±0.7	30.5±0.5
	2.5	64.0±1.4	23.3±1.6	40.7±0.5	34.4±0.5
	3.0	60.9±1.8	21.5±0.7	39.4±1.2	37.3±0.9

Data are mean ± SE

location, season, and processing methods (Hurtado-Ponce and Umezaki 1988; Ramalingam et al. 2002). For us, the shortest alkali treatment time significantly improved the gel strength, increasing it 14 times in comparison with native agar. Freile-Pelegrín and Murano (2004) found, for *G. cervicornis* (Turner) J. Agardh, a low gel strength (<50 g cm^{-2}) in native agar. They mentioned that the poor gelling ability of agarocolloids is because of the presence of D-galactose-6-sulphate residues and 4-0-methyl-α-L-galactose. The results of this study indicate that the gel strength of agar from *G. vermiculophylla* had high gel strength comparable to Superior Grade Japanese agar (Okazaki 1971).

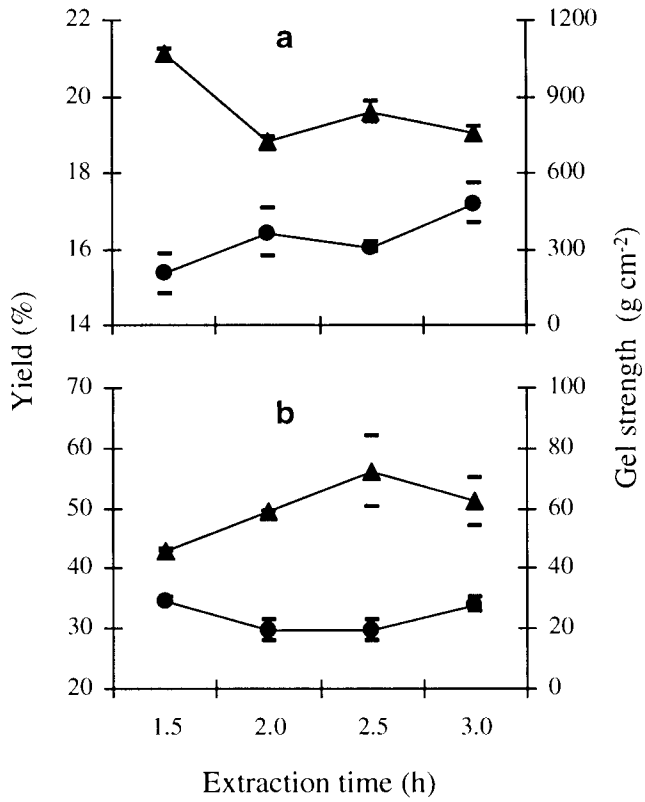

Fig. 2 Agar yield (●) and gel strength of 1.5% agar (▲) from *Gracilaria vermiculophylla* with different extraction times. **a** Alkali treated agar, **b** native agar. *Bars* represent standard error (±SE)

There was a direct relationship between the yield and gel strength with different times of alkali treatment. In contrast, during the extraction we found an inverse relation between the yield and gel strength in both native and alkali treated agar.

The alkali treated agar had a high melting temperature (92.4–99.7°C) that falls in the ranges of the United States Pharmacopeia (USP) standards (≥85°C). Melting temperatures of native agar for all extraction times (60.2–60.1°C) were lower than that measured by other authors (Freile-Pelegrín and Murano 2004; Rebello et al. 1997) in many species of *Gracilaria*.

The treatment of agar with alkali is usually done to eliminate the sulfate groups and increase the 3,6-AG content, consequently decreasing sulfation and increasing the gel strength (Chirapart et al. 1995). In our study, the native agar had lower 3,6-AG content than the alkali treated agar as a result of sulfate reduction (data not shown). Comparing the 3,6-AG content in native agar at 2 h extraction (30.5%) with the 3,6-AG of the agar with alkali treatment for the same time (44.4%), we obtained an increase of 45%. In contrast with the findings of Villanueva et al. (1997) for *G. eucheumoides* Harvey, we determined for *G. vermiculophylla* that longer times of alkali treatment reduced the 3,6-AG content.

Our results agree with Villanueva et al. (1997), confirming that optimum conditions to produce good quality agar from *Gracilaria* and other agarophytes are different from species to species, possibly caused by the variation of the specific seaweed to the treatment, hence our results may not

be applicable to other *Gracilaria* species, but they are an useful tool for agar producers.

Our results showed a significantly higher native agar yield than alkali treated agar, although the gel strength of native agar was very low (maximum 72 g cm^{-2}) and not appropriate for the food industry. After alkali treatment, the yield was reduced by 50%, but the change on gel strength increased dramatically to 1,064 g cm^{-2}. Considering the gel strength as the main criteria to process *G. vermiculophylla*, the best conditions would be as follows: an alkali treatment with 7% NaOH at 85°C, for 0.5 h.; mild acid washing with 0.025% H_2SO_4 for 2 h, and extraction of the agar in boiling water for 1.5 h. However, it is possible to increase the yield to 16.5% by increasing the extraction time to 2 h. At this time, we can obtain an agar still with commercial gel strength (700 g cm^{-2}). Under this last condition, the physical properties of the agar were: melting temperature 98°C, gelling temperature 37°C, hysteresis 60°C and 3,6-AG 44%, which are in the range expected by the food market (Armisen 1995). Considering the abundance of raw material estimated by Zertuche-González (1993), the yield and the physical properties of the agar obtained, the commercial use of *G. vermiculophylla* from Laguna San Ignacio, Baja California Sur, is well justified, under an ecological management plan.

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References

Araki C (1966) Some recent studies on the polysaccharides of agarophytes. Proc Int Seaweed Symp 5:3–19

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

Armisen R, Galatas F (1987) Production, properties and uses of agar. In: McHugh DJ (ed) Production and utilization of products from commercial seaweeds. Fish Tech Paper 288. FAO

Armisen R, Galatas F (2000) Agar. In: Phillips GO, Williams PA (eds) Handbook of hydrocolloids. CRC, Boca Raton, Fla.

Chirapart A, Katou Y, Ukeda H et al (1995) Physical and chemical properties of agar from a new member of *Gracilaria, G. lemaneiformis* (Gracilariales, Rhodophyta) in Japan. Fish Sci 61: 450–454

Duckworth M, Yaphe W (1971) The structure of agar. Part 1. Fractionation of a complex mixture of polysaccharides. Carbohydr Res 16:359–366

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

Freile-Pelegrín Y, Murano E (2004) Agars from three species of *Gracilaria* (Rhodophyta) from Yucatán Peninsula. Bioresour Technol 96:295–302

Hurtado-Ponce AQ, Umezaki I (1988) Physical properties of agar gel from *Gracilaria* (Rhodophyta) of the Philippines. Bot Mar 31: 171–174

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

Okazaki A (1971) Seaweeds and their uses in Japan. Tokai University Press

Ramalingam JR, Kaliaperumal N, Kalimuthu S (2002) Agar production from *Gracilaria* with improved qualities. Seaweed Res Utiln 24(1):25–34

Rebello J, Ohno M, Ukeda H, Sawamura M (1997) Agar quality of commercial agarophytes from different geographical origins: 1. Physical and rheological properties. J Appl Phycol 8:517–521

Santelices B (1988) Synopsis of biological data on the seaweed genera *Gelidium* and *Pterocladia* (Rhodophyta). FAO Fish Synop 145

Vergara-Rodarte MA (2006) Tendencias poblacionales de *Gracilaria vermiculophylla* (Ohmi) Papen. (Gracilariales, Rhodophyta) en la Laguna San Ignacio, B.C.S., durante un ciclo anual. Bachelor dissertation, Universidad Autónoma de Baja California Sur

Villanueva RD, Pagba CV, Montaño NE (1997) Optimized agar extraction from *Gracilaria eucheumoides* Harvey. Bot Mar 40: 369–372

Yaphe W (1984) Properties of *Gracilaria* agars. Hydrobiologia 116/117:171–186

Yaphe W, Arsenaul GP (1965) Improved resorcinol reagent for the determination of fructose and of 3,6 anhydrogalactose in polysaccharides. Anal Biochem 13:143–148

Zertuche-González JA (1993) Situación actual de la industria de las algas marinas productoras de ficocoloides en México. In: Zertuche-González JA (ed) Situación actual de la industria de macroalgas productoras de ficocoloides en América Latina y el Caribe. FAO Documento de campo No 13