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Determintion of agar gel properties of Chilean *Gracilaria* grown in the field and laboratory at different light conditions

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Abstract: The agar from the field and culture products of *Gracilaria chilensis* in Chile, were extracted by the method of Jacquline et al. (1997) and were measured on agar yield, viscosity, gelling temparature, melting temparature gel strength and flexility. The agar yield of the materials kept in the dark condition was the maximum values of 29%, while the lowest agar yield of 17% was obtained from sterile plants. The highest gel strength of 919 ± 37.5 g cm^{-2} was obtained from the cystcarpic plants, while the lowest gel strength of 444 ± 103 g cm^{-2} was obtained from sterile plants. There were not significant differences in gel strength and flexibility et at. among the materials grown in the field from different propagations and reproductive stages. The agar yiled of the materials grown without light increased, while the gel strength decreased.

Key words: *Gracilaria chilensis*, agar yield, gel properties, light condition

INTRODUCTION

Gracilaria chilensisis Bird, McLachlan and Oliveira from Chile is the world's most important source of food-grade agar (Matsuhiro & Urzua, 1990). Studies on yield and physical properties of agar from Chilean *Gracilaria* have been reported by several authors (Kim, 1970; Cote & Hanisak, 1986, Matsuhiro & Urzua, 1990; Matsuhiro *et al.*, 1992). The phenomenon of the reproductive condition dictating the type of polysaccharide elaboration in agarophytes is little known and still present conflicting results. Kim and Henriquez (1977, 1979) found differences in agar yield and gel strength between cystocarpic and tetrasporic plants, while Matsuhiro *et al.* (1992) did not find significant differences between the physical and chemical properties of agar extracted from different reproductive phases of *G. chilensis*. This study aimed to determine the agar properties of the different life phases of *G. chilensis* grown in the field and laboratory under different light conditions.

MATERIALS AND METHODS

Three samples of *Gracilaria* were collected from two sites in Chile. Three samples were collected at the River Tubul (37°14′S, 73°27′W), located 100 km to the South from Concepcion, in the Gulf of Arauco on October 29th, 1996. These *Gracilaria* samples were grown directly from spores and one by vegetative propagation. The three samples corresponded to: 1) cystocarpic plants, developed from spores growing on stones; 2) sterile plants, growing by vegetative propagation in sand, and 3) sterile plants developed from spores growing on ropes. The other three samples were collected at Coliumo Bay (36°32′S, 72°58′W) located in Dichato, at the beginning of December, 1996. The three *Gracilaria* samples collected in Coliumo were

grown for 10 days under experimental conditions with different light regimes (dark, blue and white). Blue light was obtained from three Phillips TDL 18W/18 fluorescent lamps, and white light, from three General Electric 18W fluorescent lamps with 100 ± 20 μmol photon $m^{-2}s^{-1}$. The experiments were carried out in the Marine Biological Station of the University of Concepcion, Chile. Subsamples of the dried material (100 g) were washed in tap water to remove sand and epiphytes, cut into small pieces, air-dried and then oven-dried at 70°C to a constant weight before agar extraction.

Agar extraction

Dried material of each sample was subjected to extraction following the method described by Jacquline at al. (1997). 40 g of each sample were immersed in 1.2 L of 5% NaOH solution at 80°C for 2h and washed in tap water for 30 min. to remove the excess NaOH. The alkali treated samples were neutralized in 1 L of 1.5% H_2SO_4 solution for 1h and washed in tap water for another 12h. The agar was extracted by boiling the samples in 1.2 L of distilled water for 2h. The extracted agar was filtered through a vacuum pump equipped with a Buchner Funnel no 6 with 3 m pore size industrial paper (Advantec, Toyo Roshi Co., Japan) and kept at room temperature until gel formation. The agar gel was sliced, frozen at -35°C for 24h, thawed in tap water, air-dried and then oven-dried at 40°C for determination of agar yield. Viscosities of the solution at 80°C were determined using a Brookfield Viscometer (BL- no 1 spindle at 60 rpm, Tokyo Keiki). Gelling temperature was determined according to Kim (1970) and melting temperature was measured as described by Hurtado-Ponce and Umezaki (1988). Three observations were made for each sample.

Rheological measurements

Gel texture was measured using a Sun-Rheometer CR-200D equipped with a cylindrical plunger of 1 cm^2 diameter operating at a maximum force of 2 kg and table speed of 20 mm min^{-1}. The load deformation curve were derived from the rheological parameters which were defined as gel strength, hardness and flexibility. All measurements of the physical gel properties were done on 1.5% agar solution after being stabilized for 15h at 20°C using three replicates for each sample.

RESULTS AND DISCUSSION

Differences in agar yield, viscosity and gel strength of *Gracilaria chilensis* were observed among the plants grown in the field, from different propagations and reproductive stages. Maximum agar yield of 29% was obtained by the plants grown without light, in a controlled environment condition, while the lowest agar yield of 17% were obtained from sterile plants developed from spores, grown on ropes in the field (Table 1). Gelling and melting temperatures showed little differences between the plants developed from different propagations and reproductive stages. High gelling temperatures (55-56°C) and low melting temperatures (78-82°C) were observed among the agar extracts. In this study, the gelling and melting temperatures did not meet the United States Pharmacopeia (USP) standards which require that agars should have gelling temperatures between 32-39°C and that they should not melt below 85°C.

The highest viscosity of 18.1 ± 0.4 cP was obtained from sterile plants, grown by vegetative propagation in sand, while the lowest viscosity of 9.5 ± 0.3 cP was obtained from the sterile plants developed from spores grown on ropes. Little differences were observed in viscosities between the plants grown under different light conditions. The plants grown under white light gave an agar with high viscosity (16.8 ± 0.6 cP), while the lowest viscosity was produced from plants grown without light (12.5 ± 0.3 cP).

Table 1. Yield and physical properties of 1.5% agar obtained from Chilean *Gracilaria* grown in the field and at different light conditions.

Samples	Agar yield %	Viscosity cP	Gelling temperature °C	Melting temperature °C
Samples grown in the field				
1	26	13.8±0.4	55±1.0	80±1.7
2	25	18.1±0.4	56±1.2	82±1.7
3	17	9.5±0.3	56±0.6	80±0.6
Samples grown during 10 days under different light conditions				
4	29	12.5±0.3	56±0.3	80±0.6
5	24	13.0±0.1	55±1.0	80±1.5
6	27	16.8±0.6	56±0.6	78±3.0

1 - cystocarpic plant developed from spores, grown on stones
2 - sterile plant grown by vegetative propagation in sand
3 - sterile plant developed from spores grown on ropes
4 - plant grown without light (dark)
5 - plant grown under blue light
6 - plant grown under white light

Table 2. Gel strength and Flexibility of 1.5% agar from Chilean *Gracilaria* grown in the field and at different light conditions.

Samples	Gel strengh g. cm^{-2}	Flexibility g mm x 10^2
Samples grown in the field		
1	919±38	2.7 ±0.1
2	444±103	1.92±1.6
3	605	1.10
Samples grown during 10 days under different light conditions		
4	498±77	2.1 ±1.5
5	542±28	1.60±0.2
6	627±28	1.8 ±0.3

1 - cystocarpic plant developed from spores, grown on stones
2 - sterile plant grown by vegetative propagation in sand
3 - sterile plant developed from spores grown on ropes
4 - plant grown without light (dark)
5 - plant grown under blue light
6 - plant grown under white light

Gel strength and flexibility are shown in Table 2. Among the plants grown in the field, the highest gel strength of 919±37.5 g cm^{-2} and the most flexible gel (2.73±0.08 g mm x 10^2) were obtained from the cystocarpic plants developed from spores, while the lowest gel strength of 444±103 g cm^{-2} was obtained from sterile plants grown by vegetative propagation. These results agree with those of Whyte *et al.* (1981) for G. *verrucosa*, who found that the quality of agar depends on the life stage of the alga, decreasing in order of merit from the cystocarpic to the vegetative plants. The high gel strength obtained from *G. chilensis* in this study

is similar to the gel strength of this species reported by Kim (1970). There are no significant differences in gel strength and flexibility of agars from the plants grown under the different light conditions. A slightly high gel strength of 627 ± 28 g cm^{-2}, was obtained from plants grown under white light regime and the most flexible gel (2.13 ± 1.5 g mm x 10^2) from plants grown without light.

The response of *G. chilensis* to the light quality and dark treatment agrees well with information from the literature. The high values of agar yield in plants that were exposed to dark correspond to the effect observed in *Gracilaria* sp. (Yu and Pedersen, 1991) and *Gracilariopsis lemaneiformis* (Rincones *et al.*, 1993), while the lowest values of agar production in plants growing under blue light, also agree well with those in other algae as given by Clauss (1970), McLear (1986) and Kowallik (1987). The mechanisms involved in those phenomena are not well known yet, however, since metabolic reactions are catalyzed by enzymes, these responses could be a result of regulation by light quality (Ryters, 1987).

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REFERENCES

CLAUSS, H., 1970. Effect of the red and blue light on morphogenesis and metabolism in *Acetabularia mediterranea*. In Brachet, J. and Bonotto, S., (eds.), Biology of *Acetabularia*, Academic Press, New York, 177-191.

COT, G.L, HANISAK, M.D., 1986. Production and properties of native agar from *Gracilaria tikvahiae* and other red algae. Bot. Mar. **29**: 359-366.

HAYASHI, K. and A. OKAZAKI, 1970. Handbook on Agar. Korinshoin, Tokyo. 534 pp. (in Japanese).

HURTADO-PONCE, A.Q., I. UMEZAKI, 1988. Physical properties of agar gel from *Gracilaria* (Rhodophyta) of the Philippines. Bot. Mar. **31**: 171-174.

REBELLOM, J., M. OHNO, H. UKETA, M. SAWAMURA, 1997. Agar quality of commercial agarophytes from different geographical origines: 1. Physical and rheological properties. J. Applied Phycol. 8: 517-521.

KIM, D.H., 1970. Economically important seaweeds in Chile. I. *Gracilaria*. Bot. Mar. **13**: 140-162.

KIM, D.H., HENRIQUEZ, P., 1977. Agar-agar from cystocarpic and tetrasporic plants of *Gracilaria verrucosa*. I. Yield and gel strengths. J. Phycol., **13**: supl. 35.

KIM, D.H., HENRIIQUEZ, P., 1979. Yields and gel strength of agar from cystocarpic and tetrasporic plants of *Gracilaria verrucosa* (Florideophyceae) in Jensen A, Stein JS, (eds.), Proceedings of the Ninth International Seaweed Symposium, Science Press, Princepton, **9**: 257-262.

KOWALLIK, W., 1987. Blue light effect on carbohydrate and protein metabolism. In H. Senger, (ed.), Blue Light Responses: Phenomena and Occurrence in Plants and Microorganisms, Vol. I, CRC Press, Boca Raton, Florida, pp. 7-16.

MATSUHIRO, B., URZUA, C.C., 1990. Agars from *Gracilaria chilensis* (Gracilariales) J. appl. Phycol., **2**: 273-279.

MATSUHIRO, B., RIVAS, P., LAMBA, D., 1992. Polisacaridos de las fases nucleares de *Gracilaria chilensis*. *Bol. Soc. Chil. Quim.*, **34**: 89-95.

MCLEAR, B.A., 1986. Regulation of carbon flow by nitrogen and light in the red algae, *Gelidium coulteri*. Plant Physiol., **82**: 136-141.

RINCONES, R., Y. SHUKUN and M. PEDERSEN, 1993. Effect of dark treatment on the starch degradation and the agar quality of cultivated *Gracilariopsis lemaneiformis* (Rhodophyta, Gracilariales) from Venezuela. Hydrobiologia, **260/261**: 633-640.

RUYTERS, G., 1987. Control of enzyme capacity and enzyme activity. In H. Senger, (ed.), Blue Light Responses: Phenomena and Occurrence in Plants and Microorganisms, Vol. II, CRC Press, Boca Raton, Florida, 71-88.

WHYTE, J.N.C., J.R. ENGLAR, R.G. SAUNDERS and J.C. LINDSAY, 1981. Seasonal variations in the biomass, quantity and quality of agar, from the reproductive and vegetative stages of *Gracilaria* (*verrucosa* type). Bot. Mar., **24**: 493-501.

YU, S. and M. PEDERSEN, 1991. Carbon partitioning in red algae. Influence of ammonia, NH_4^+/NO_3^-, salinity and darkness on the carbon flow into cell polymers, floridean starch and floridoside. In G. Garcia-Reina and M. Pedersen (eds.), Proc. Cost. (Sub-group 1). Workshop on Seaweed Cellular Biotechnology, Physiology and Intensive Cultivation. Las Palmas, Spain, pp. 167-184.

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