

## Seasonal changes in agar characteristics of two populations of *Pterocladia capillacea* in Gran Canaria, Spain

Y. Freile-Pelegri<sup>1,2,\*</sup>, D. Robledo<sup>2</sup>, R. Armisén<sup>3</sup> & G. García-Reina<sup>1</sup>

<sup>1</sup>Instituto de Algología Aplicada, Box 550, Las Palmas de Gran Canaria, Spain

<sup>2</sup>CINVESTAV-Unidad Mérida, AP. 73 Cordemex 97310, Mérida, Yucatán, México

<sup>3</sup>HISPANAGAR, SA. AP. 329-09080 Burgos, Spain

(E-mail: freile@kin.cieamer.conacyt.mx)

(\* Author for correspondence; present address)

**Key words:** *Pterocladia capillacea*, agar, seasonality

### Abstract

Agar characteristics of *Pterocladia capillacea* were examined seasonally at two intertidal populations exposed to different wave energy on the northern rocky shore of Gran Canaria Island. Plants were collected monthly from August 1991 to July 1992. Agar yield, gel strength, melting and gelling temperature and chemical properties such as sulphate and pyruvate content were measured. Percent epiphytism was determined on both populations, together with the changes in biomass as dry weight. Specimens in the sheltered habitat were larger and more epiphytized than ones in the exposed area. There was a clear seasonal change in agar characteristics in both populations. Agar yields decreased in late spring and early summer, although in the sheltered habitat fluctuations were more erratic. Gel strength increased in winter, reaching a maximum in December-February. No significant differences were found in agar yield, gel strength or melting and gelling temperatures, but there was a difference between fish to dry weight ratio. The role of the exposure degree as a possible environmental factor responsible for this behavior is discussed. Agars of *Pterocladia capillacea* from Canary Islands show characteristics for industrial use.

### Introduction

Agars are polysaccharides in the intercellular matrix of primarily two red algal families, Gracilariaceae and Gelidiaceae (Craigie, 1990). Agar provides structural support in response to water movements resulting in elasticity and rigidity of the alga. The structure of agar is basically composed of neutral and charged galactoses consisting of alternating molecules of D-galactose and 3,6 anhydro-L-galactose (Duckworth & Yaphe, 1971). Charged residues such as sulphate esters and pyruvate acetal play an important role in the physical and rheological properties of agar.

*Pterocladia* spp. are of considerable commercial significance as a source of the phycocolloid agar. They are the next important source of bacteriological agar and agarose to *Gelidium* (Armisen & Galatas, 1987). Both genera are exploited in the Azores and in New

Zealand for the agar industry (McHugh, 1991). *Pterocladia* includes 10–12 tropical species having only two species common to temperate waters, *P. capillacea* (Gmelin) Bornet et Thuret and *P. lucida* (Turner) J. Agardh. The temperate species grow where there is strong water motion, which is considered to be the main factor affecting shore plant distribution (Santelices, 1988). Survival of this macroalga depends on its ability to withstand the hydrodynamic forces generated by breaking waves, an ability that may be related to both the morphology and size of the plant.

Various factors such as habitat, water temperature, light intensity and geography as well as biotic interactions such as epiphytism influence the relative proportions of seaweed constituents (Santelices, 1988). The effect of environmental conditions on agar composition is usually studied by following seasonal variations in quantity and quality. These variations may be regarded

as biological alterations of the chemical and physical properties of the cell wall to meet environmental or physiological demands (Craigie & Wen, 1984). Several authors have reported these changes in other seaweed species, with only limited data for *Pterocladia capillacea* (Friedlander & Zelikovitch, 1984; Oliveira et al., 1995) (Table 1).

The influence of local or micro environmental factors on seasonal changes in phycocolloid yields and properties are often not known or inadequately documented (Craigie, 1990). Correlations have been found between species zonation, ecological distribution, and cell wall composition suggesting that matrix polysaccharides such as agar may be involved in mechanical regulations (Kloareg & Quatrano, 1988). For example, in *Mastocarpus stellatus* and *Chondrus crispus* Dudgeon and Johnson (1992) found that differences in mechanical properties of the stipe may be reflected in differences of plant size which could be explained as different cell wall polysaccharides composition; however, they did not evaluate polysaccharides of these species.

Aim of this study was to present the seasonal differences in yield, rheological and physico-chemical properties of agar from *Pterocladia capillacea*.

## Materials and methods

### Study area

The Canary Islands are volcanic in origin with a rocky coastline consisting mostly of weathered basalt. The Islands are bathed by the relatively cold water of the Canary current flowing from NNE with surface temperatures at Gran Canaria between 18 and 23 °C. Salinity in oceanic waters around Canary Islands is stable at 37‰. Tidal range is moderate with a mean high tide level of 2 m and a mean low tide level of 0.8 m. In the northern rocky coast of the Canary Islands, *Pterocladia capillacea* is commonly found in the middle to low intertidal zone forming dense patches. Two localities exhibiting different wave exposure degree were sampled monthly. Quantitative measurements of water movement applicable to benthic situations are difficult to apply in wave beaten habitats; therefore the wave exposure was described in terms of prevailing currents, wind direction and coastal topography. Bocabarranco at the north (28°09'N, 15°40'W) is a sheltered zone protected from direct wave action by rock outcroppings while Agaete at the northwest (28°06'N, 15°43'W) is

a more exposed boulder site directly impacted by the waves from the open coast.

### Plant collection

*Pterocladia capillacea* plants were collected from August 1991 to July 1992 together with measurements of water temperature. Plants were cut above the holdfast during the lowest tides of each month. In the laboratory they were washed thoroughly with tap water to remove silt and sand. Wet weight was measured to the nearest 0.01 g after centrifugation in a commercial laundry centrifuge for 10 s to remove excess water. 100 g wet material was weighed ( $n = 3$ ), oven dried overnight at 60–70 °C and reweighed. Samples were stored in sealed plastic bags until agar extraction.

To determine percent of epiphytism on *Pterocladia capillacea*, three sub-samples from the fresh material were weighed. Epiphytes were cleaned by brushing and scrapping *Pterocladia* fronds. The weight recorded after this procedure was taken as the value of pure seaweed (percent of agarophyte from original samples). Three samples (1 g each) of fresh pure seaweed were oven dried for 24 h at 60 °C to estimate monthly biomass changes in terms of dry weight.

### Agar extraction

Dry seaweeds were exposed to a 0.5% solution of Na<sub>2</sub>CO<sub>3</sub> at 85–90 °C for 30 min prior to extraction and washed with running tap water for 10 min. Agar was extracted ( $n = 3$ ) with distilled water at pH between 6.0–6.5 and autoclaved at 120 °C for 2 h. The mixture was ground with a commercial blender and heated at 90 °C with diatomaceous earth for 30 min and finally pressure filtered (Armisen & Galatas, 1987). The filtrate was allowed to gel at room temperature, frozen overnight and thawed. Finally the agar was oven dried for 24 h at 60 °C, cooled and weighed to calculate percent agar yields.

### Gel properties

Dry agar was ground in a Tecator mill and reconstituted into 1.5% w/v solutions to measure physico-chemical characteristics (gel strength, melting and gelling temperature,  $n = 3$ ). Gel strength was measured after gelling overnight at room temperature by measuring the load (g cm<sup>-2</sup>) causing the cylindrical plunger (1 cm<sup>2</sup> cross-section) to break a standard gel in 20 s (Armisen & Galatas, 1987).

Table 1. *Pterocladia capillacea*. Agar content physical properties and chemical characteristics found by other authors at different locations.

Locality	Agar content (%)	Gel strength (g cm <sup>-2</sup> )	Gelling T (°C)	Melting T (°C)	Sulphate (%)	Pyruvate (%)	Source
Barbados	15.3 <sup>a</sup>	--	--	--	3.7	0.65	Young et al. (1971)
Egypt	14.0–27.0 <sup>a</sup>	--	--	--	--	--	Rao & Bekheet (1976)
Brazil	36.5–37.0	--	--	--	--	--	Santos (1980)
Hawai	28.9–31.1	--	--	--	--	--	Santos (1980)
Florida	33.0–41.0 <sup>a</sup>	110–190	41.0–45.0 <sup>a</sup>	82.0–85.0 <sup>a</sup>	1.80–2.20	--	Cote & Hanisak (1986)
Israel	28.5 <sup>a</sup>	448 <sup>a, b</sup>	27.7	98	0.48	--	Friedlander & Lipkin (1982)
Israel	5.5–32.0 <sup>a</sup>	150–950 <sup>a, b</sup>	21.0–32.0 <sup>a, b</sup>	91.0–99.0 <sup>a, b</sup>	1.20–2.50 <sup>a</sup>	--	Friedlander & Zelikovitch (1984)
Venezuela	12.4–27.6	1313–1470	35.0–35.5	97	0.30–2.60	0.0	Lemus et al. (1991)
Brazil	15.0–34.0	--	--	--	--	--	Oliveira & Berchez (1993)
Brazil	5.5–32.2	--	--	--	1.00–4.60	--	Oliveira et al. (1995)
Spain	15.0–29.5 <sup>a</sup>	813–1428 <sup>a</sup>	34.6–36.3 <sup>a</sup>	88.9–95.5 <sup>a</sup>	2.12–3.43 <sup>a</sup>	0.14–0.47 <sup>a</sup>	This study (Agarte)
Spain	16.7–29.8 <sup>a</sup>	912–1354 <sup>a</sup>	34.4–36.9 <sup>a</sup>	86.9–94.9 <sup>a</sup>	1.98–3.10 <sup>a</sup>	0.12–0.50 <sup>a</sup>	This study (Bocabarranco)

<sup>a</sup>Native agar (without alkali treatment);

<sup>b</sup>1% agar solutions.

Gelling temperature was obtained by the addition of 10 ml hot agar solution into a test tube (2.3 cm diameter, 6 cm height). A glass bead (5 mm diameter) was placed in the test tube. The tube was tilted up and down in a water bath at room temperature until the glass bead ceased moving. The gel temperature in the tube was immediately measured introducing a precision thermometer (0.1 °C divisions). Melting temperature of the gel in a test tube (2.3 cm diameter, 16.5 cm height) was measured by placing an iron bead (9 mm diameter) on the gel surface. The test tube was clamped in a water-bath and the temperature raised from 50 to 100 °C; melting point was recorded with a precision thermometer when the bead sank into the solution.

#### Agar substitutions

Percent sulphate was determined by hydrolyzing 1 g of agar powder (previously dried at 105 °C) in 10 ml HNO<sub>3</sub> in 100 ml Kjeldahl flasks that results in complete hydrolysis of the ester sulphate followed by quantitative precipitation with barium chloride of the liberated sulphate. The precipitates were collected on ash-free gravimetric filters, dried, ignited and weighed on a precision balance (0.0001 g). The weight of the obtained barium sulphate, multiplied by 0.4116, gave the equivalent of sulphate. Percent pyruvate was determined by hydrolyzing 0.5 g of agar powder in oxalic acid 0.02 M following the spectrophotometric method based on lactic dehydrogenase by Duckworth & Yaphe (1970).

#### Statistical analysis

The data were tested for normality (Kolmogorov-Smirnov) using a statistical software package (Stasoft). Data were subjected to the Bartlett's test for homogeneity of group variances and to Pearson's product moment correlation test to determine linear relationship between treatments. Spearman correlation was used when necessary. Multifactorial analyses of variance were applied to determine the effects of single treatments and degrees of interaction between different treatments on agar characteristics whenever data groups exhibited homogeneity. The single and combined effects of season and locality on agar properties for *Pterocladia capillacea* were determined as well through multifactorial analysis of variance. MANOVA comparison of means was done using Tukey's HSD test. All heterogeneous data groups were transformed by different methods including arcsin square root of  $x$ ,  $\log(x + 1)$  and  $\ln(x + 1)$  in order to produce the homogeneity required for a multifactorial analysis of variance. Monthly mean differences for data groups that retained their heterogeneous character were tested using non-parametric Kruskal-Wallis one way analyses of variance.

#### Results

*Pterocladia capillacea* grows year round in the two study areas on the northern rocky shore of Gran

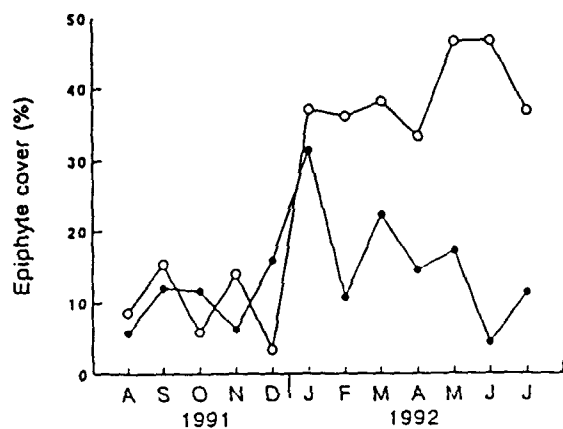


Figure 1. Epiphytic coverage on samples collected at the exposed Agaete site (•) and the sheltered Bocabarranco site (○) during the study period. Bars represents standard deviation.

Canaria. Specimens collected in Agaete were shorter, forming dense mats while in Bocabarranco were larger and quantitatively more epiphyted throughout the year. The mean value for epiphyte coverage was 26.6% of the fresh weight in Bocabarranco, whereas in Agaete only 13.6% of plant fresh weight was from epiphytes. In Bocabarranco the increase in epiphyte cover was more evident from late winter to the beginning of summer with the maximum value between May–June (46.8%) while in Agaete the maximum was recorded in January, 31.4% (Figure 1). The most common epiphyte was the crustose red alga *Lithothamnion* sp. although during the summer months *Ulva rigida* was most evident coinciding with an increase in water temperature.

Fluctuations in percent dry weight followed a seasonal pattern. In both localities the maximum values were found in early autumn, September in Agaete and October in Bocabarranco, and minimum in winter and spring (Figure 2). The mean value in Agaete (28.6%) was higher than in Bocabarranco (25.8%).

Sea water temperature ranged between 17.7 and 23.5 °C with maximum values recorded during late summer and early autumn (Figure 2). There was a positive correlation between sea water temperature and dry weight in Agaete ( $r = 0.36$ ,  $p < 0.05$ ) and in Bocabarranco ( $r = 0.52$ ,  $p < 0.05$ ). No significant correlation was found between sea water temperature and agar yield at Agaete ( $r = 0.23$ ) or Bocabarranco ( $r = 0.29$ ).

Agar content ranged from 15% to 29.5% in Agaete and from 16.7% to 29.8% in Bocabarranco (Figure 3a). Agar content was slightly higher in Agaete (22.9%)

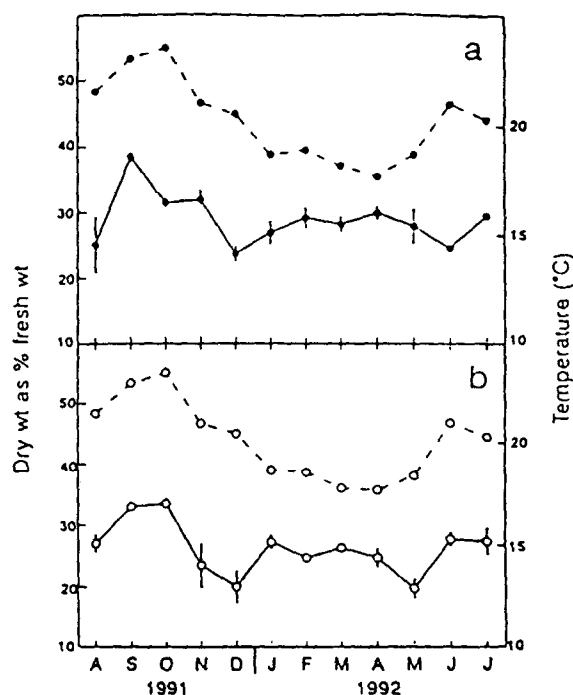


Figure 2. Dry weight fluctuations (—) in relation to seawater temperature (---) in (a) Agaete and (b) Bocabarranco. Bars represents standard deviation.

than in Bocabarranco (21.4%). Overall, the highest yields were obtained from plants harvested during late summer and autumn, with a maximum value in August for Bocabarranco, whereas in Agaete the highest peak value was found in December. In both localities, agar yields declined in late spring and early summer (Figure 3a). There was a positive correlation between agar yield values in Agaete and Bocabarranco ( $r = 0.35$ ,  $p < 0.05$ ).

Gel strength ranged from 813 to 1428 g cm<sup>-2</sup> for Agaete (mean, 1145 g cm<sup>-2</sup>) and between 912 to 1354 g cm<sup>-2</sup> (mean, 1170 g cm<sup>-2</sup>) for Bocabarranco. In both localities it increased in winter, reaching a maximum in December–February (Figure 3b). Minimum values were found in August at Bocabarranco, and in November at Agaete. There was a positive correlation ( $r = 0.53$ ,  $p < 0.01$ ) in this variable between the sites.

In both localities, the sulphate content was highest in March 3.43% in Agaete and 3.11% in Bocabarranco (Figure 3c), and was positively correlated between both sites ( $r = 0.59$ ,  $p < 0.05$ ). There was no correlation between gel strength and sulphate content in either site (Table 2). Percent of pyruvic acid ranged from

Table 2. Correlation coefficient matrix of various chemical and physical properties of agar characteristics sampled in this study. Correlation coefficients greater than 0.36 are significant at the 95% level ( $p < 0.05$ ).

Agate/Bocabarranco	Gelling temperature (°C)	Melting temperature (°C)	Gel strength (g cm <sup>-2</sup> )	Sulphate content (%)
Melting temperature (°C)	0.36/0.55	--	--	--
Gel strength (g cm <sup>-2</sup> )	-0.21/0.26	0.36/0.44	--	--
Sulphate content (%)	-0.24/-0.32	-0.13/-0.42	0.18/-0.01	--
Agar content (%)	0.43/0.29	0.34/0.22	0.15/-0.37	-0.30/0.13

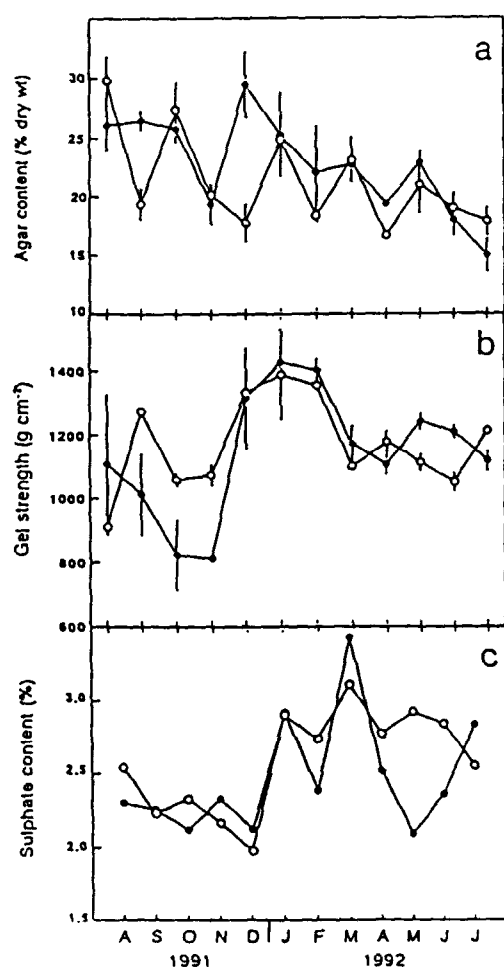


Figure 3. Seasonal variation of the agar characteristics at Agaete (+) and Bocabarranco (o). a. Agar yield expressed as percent of dry weight from pure seaweed. b. Gel strength of 1.5% agar solution. c. Sulphate content in agar samples. Bars represent standard deviation.

0.14% to 0.47% in Agaete and from 0.12% to 0.50% in Bocabarranco.

Mean gelling temperatures were 35.4 and 35.6 °C for Agaete and Bocabarranco respectively, while melting temperature was 91.9 and 91.6 °C, respectively (Table 3). There was a positive correlation for gelling temperature between Agaete and Bocabarranco ( $r = 0.47$ ,  $p < 0.01$ ) and for melting temperatures between both sites ( $r = 0.69$ ,  $p < 0.01$ ). Data showed a positive correlation between gel strength and melting temperature in Agaete and Bocabarranco (Table 2).

Gel strength data showed monthly significant differences when analyzed by the MANOVA test. However, no significant differences between Agaete and Bocabarranco were found for this variable. Due to the heterogeneity of variance data for agar content, dry weight, gelling and melting temperatures were analyzed using Kruskal Wallis one way analysis of variances. This showed monthly significant differences ( $p < 0.01$ ), and a significant difference between localities for the dry weight ( $p < 0.01$ ) (Table 4).

## Discussion

Two main findings were obtained from this study. Agar characteristics changed seasonally in both *Pterocladia capillacea* populations, agar yield decreased in late spring and gel strength increased in winter. No evidence of influence of wave exposure degree on the agar characteristics was found, although differences between localities were found in plant size, dry weight and epiphyte cover.

The seasonal pattern of agar yield showed a more erratic fluctuation over the year in the sheltered intertidal population at Bocabarranco. Although few data exist in the literature for the agar in *Pterocladia capillacea* comparisons on agar yield values were similar

Table 3. Gelling and melting temperatures of agar at both localities.

Month	Agaete		Bocabarranco	
	Gelling T°C	Melting T°C	Gelling T°C	Melting T°C
Aug	36.0±0.5	92.6±1.0	35.7±0.6	94.2±0.3
Sep	36.3±0.2	89.2±1.1	34.7±0.3	93.7±0.6
Oct	35.9±0.6	93.5±0.5	36.6±0.6	92.9±0.8
Nov	35.4±0.5	93.9±0.7	36.4±0.4	92.7±0.7
Dec	35.4±0.2	93.2±0.8	36.1±0.0	93.8±0.6
Jan	35.6±0.3	95.5±0.3	36.9±0.8	93.7±0.2
Feb	35.6±0.1	94.6±1.0	36.5±0.3	94.9±0.1
Mar	34.6±0.1	88.9±0.0	34.4±0.3	88.7±0.5
Apr	35.1±0.3	89.2±0.0	35.1±0.0	89.8±0.8
May	35.4±0.5	90.2±0.0	34.8±0.3	89.4±0.3
Jun	35.3±0.2	91.1±0.6	35.0±0.0	86.9±0.4
Jul	34.9±0.1	89.5±0.4	34.7±0.7	88.3±0.3

to those obtained by others (Table 1). Minimum values were found in early summer coinciding with the lowest values found by other authors in the same months (Friedlander & Zelikovitch, 1984; Oliveira & Berchez, 1993; Oliveira et al., 1995). However, in this study agar yield evaluations were based on dry seaweed free of epiphytes that gives more reliable yield values.

Dry to wet weight ratio may reflect many physiological and biochemical processes within the alga, such as storage of high molecular weight reserves (Craigie, 1990). Although no significant correlation between dry weight and agar yield were found in both populations, the highest values of dry weight and agar yield occurs in the same season. The increase in biomass based on dry weight changes was related to an increase in seawater temperature. It appears that an increase in dry weight was stimulated in autumn by conditions of high temperature and high light intensity (see also Fralick & Andrade, 1981) for populations at the Azores. The mean dry weight values for *Pterocladia capillacea* in the Canary Islands were higher than those obtained by Friedlander & Lipkin (1982) in the Mediterranean (24.8%).

The difference between the two localities in dry weight values may be related to plant size. The morphological variations observed in *Pterocladia capillacea* between sheltered and exposed population can be explained by differences in water movement. Similar effects of wave exposure on morphology in *P. capillacea* have been observed in Brazil (Oliveira & Berchez, 1993) and morphological variations in *P. caerulea* in Hawaii (Santelices, 1978).

Epiphytism also seems to be affected by wave exposure. Lowest epiphytism was found during autumn associated with higher tides and increased water movement (Figure 1). In Agaete epiphytism showed a more homogeneous pattern throughout the year most probably due to higher exposure. In addition, epiphyte coverage may have affected dry weight values. According to Santelices (1988) epiphytes are particularly common on larger subtidal thalli, reducing the amount of available light and decreasing the agar yield. In this regard, Torres et al. (1991) showed a direct relationship between photosynthetic rates and carbon and nitrogen content in *Gelidium sesquipedale*, with a reduction in the C:N ratio under decreasing irradiance and leading to a decrease in cell-wall polysaccharides. Perhaps the small differences in agar yield between the sites in this study are due to differences in epiphyte cover.

Oliveira et al. (1995) found a higher agar content in *P. capillacea* plants inhabiting more turbulent waters in Brazil. In the present study there was no evidence for this, although the values of agar yield and gel strength were slightly higher in the more exposed intertidal population (Agaete). However, it is difficult to compare our study in Gran Canaria with that of Brazil because the factors involved in seasonal effects are likely to differ from one location to another.

Gel strength followed a similar seasonal pattern in both localities in this 12-month study. Friedlander & Zelikovitch (1984) showed a different gel strength pattern in a 7-month study, with a maximum in summer (950 g cm<sup>-2</sup>). Our gel strength values are comparable with those obtained by Lemus et al. (1991), although no alkali treatment was performed. Thus, the gel strength

Table 4. Kruskal-Wallis ANOVA test for significance differences between the two localities in agar characteristics and dry weight.

Variable	n	Sum of ranks	H	p
<b>Agar yield</b>				
Agacete	32	1206.0	2.194	0.138
Bocabarranco	35	1072.0		
<b>Dry weight</b>				
Agacete	32	1237.0	7.057	<0.01
Bocabarranco	32	834.0		
<b>Gelling temperature</b>				
Agacete	33	1086.5	0.198	0.655
Bocabarranco	34	1191.5		
<b>Melting temperature</b>				
Agacete	31	1050.5	0.647	0.421
Bocabarranco	32	965.5		

values obtained here are higher than previous studies (Table 1).

There was no correlation between sulphate and gel strength as stated by Yaphe & Duckworth (1972). During alkali treatment the L-galactose 6-sulfate can be converted to 3,6-anhydro L-galactose, however the low concentration of  $\text{Na}_2\text{CO}_3$  would not hydrolyze sulphate in this position and would not affect the chemical composition of native agar. Similar to our results, Mouradi-Givernaud et al. (1992) could not find any correlation between the sulphate content and gel strength in *Gelidium latifolium*.

Pyruvate content was low when compared to the other authors (Table 1), however, values should be taken with caution since absolute values for the pyruvate content of agar can only be quantified with the use of nuclear magnetic resonance spectroscopy (NMR).

Any correlation between gel strength and melting temperature can in part be explained by the methoxyl substitutions at various position in the agar molecule (Yaphe & Duckworth, 1972). High values of gelling and melting temperatures of the agar in *Pterocladia capillacea* corresponds with high gel hysteresis, thus gel strength is the most important parameter used to determine applications of agar.

In the genus *Pterocladia* gel strengths above 700  $\text{g cm}^{-2}$  and low sulphate and pyruvate values (below 5% and 0.5% respectively) is critical for bacteriological uses (Armisen & Galatas, 1987). Our results show that agar from *Pterocladia capillacea* in Gran Canaria has commercial value.

## Acknowledgements

This study was supported by a fellowship from Fundación Universitaria de Las Palmas to Y. Freile-Pelegrín. CONACYT (211085-54956T) is acknowledge for financial support to D. Robledo. The authors thank Prof. L. Capurro for linguistic advance and making constructive comments to the manuscript.

## References

- Armisen R, Galatas F (1987) Production, properties and uses of agar. In: McHugh DJ (ed.), Production and utilization of products from commercial seaweeds. FAO Fish. Tech. Pap. 288: 1-57.
- Cote GL, Hanisak MD (1986) Production and properties of native agars from *Gracilaria tikvahiae* and other red algae. Bot. mar. 29: 359-366.
- Craigie J (1990) Cell wall. In: Cole KM, Sheath RG (eds), Biology of the Red Algae. Cambridge University Press: 227-257.
- Craigie JS, Wen ZC (1984) Effects of temperature and tissue age on gel strength and composition of agar from *Gracilaria tikvahiae* (Rhodophyceae). Can. J. Bot. 62: 1665-1670.
- Denny MW (1988) Biology and mechanics of the wave-swept environment. Princeton University Press, Princeton, New Jersey: 320.
- Duckworth M, Yaphe W (1970) Definitive assay for pyruvic acid in agar and other algal polysaccharides. Chem. Ind. (London) 23: 747-748.
- Duckworth M, Yaphe W (1971) The structure of agar. Part 1: Fractionation of a complex mixture of polysaccharides. Carbohydr. Res. 16: 189-197.
- Dudgeon SR, Johnson AS (1992) Thick vs. thin: thallus morphology and tissue mechanics influence differential drag and dislodgment of two co-dominant seaweeds. J. exp. Mar. Biol. Ecol. 165: 23-43.
- Fralick RA, Andrade F (1981) The growth, reproduction, harvesting and management of *Pterocladia pinnata* (Rhodophyceae) in the Azores, Portugal. Proc. 10th Int. Seaweed Symp.: 643-648.
- Friedlander M, Lipkin Y (1982) Rearing of agarophytes and carrageenophytes under field conditions in the eastern Mediterranean. Bot. mar. 25: 102-105.
- Friedlander M, Zelikovitch N (1984) Growth rates, phycocolloid yield and quality of the red seaweeds, *Gracilaria* sp., *Pterocladia capillacea*, *Hypnea musciformis*, and *Hypnea cornuta*, in field studies in Israel. Aquaculture 40: 57-66.
- Haug A, Larsen B (1974) Biosynthesis of algal polysaccharides. In: Pridham JB (ed.), Plant Carbohydrate Biochemistry. Academic Press New York: 207-218.
- Kloareg B, Quatrano RS (1988) Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. Oceanogr. Mar. Biol. Annu. Rev. 26: 259-315.
- Kraemer GP, Chapman DJ (1991) Biomchanics and alginic acid composition during hydrodynamic adaptation by *Egregia menziesii* (Phaeophyta) juveniles. J. Phycol. 27: 47-53.
- Lemus A, Bird K, Kapraun DF, Koehn F (1991) Agar yield, quality and standing crop biomass of *Gelidium floridanum* and *Pterocladia capillacea* in Venezuela. Food Hydrocolloids 5: 469-479.
- McHugh DJ (1991) Worldwide distribution of commercial resources of seaweeds including *Gelidium*. Hydrobiologia 221: 19-29.

- Mouradi-Givernaud A, Givernaud T, Morvan H, Cosson J (1992) Agar from *Gelidium latifolium* (Rhodophyceae, Gelidiales): Biochemical composition and seasonal variations. *Bot. mar.* 35: 153–159.
- Oliveira EC, Berchez FAS (1993) Resource biology of *Pterocladia capillacea* (Gelidiales, Rhodophyta) populations in Brazil. *Hydrobiologia* 260/261: 255–261.
- Oliveira EC, Saito R, Santos Neto JF, Garófalo GMC (1995) Temporal and spatial variation of agar from a population of *Pterocladia capillacea* (Gelidiales, Rhodophyta) from Brazil. XVth International Seaweed Symposium (abstracts).
- Rao AV, Bekheet IA (1976) Preparation of agar-agar from the red seaweed *Pterocladia capillacea* of the coast of Alexandria, Egypt. *Apl. envir. Microbiol.* 32: 479–482.
- Santelices B (1978) The morphological variation of *Pterocladia caerulescens* (Gelidiales, Rhodophyta) in Hawaii. *Phycologia* 17: 53–60.
- Santelices B (1988) Synopsis of biological data on the seaweed genera *Gelidium* and (Rhodophyta). *FAO Fish. Synop.* 14: 55.
- Santos G (1980) Quality of carrageenan and agar. In: Abbott IA, Foster MS, Eklund LF (eds), *Pacific Seaweed Aquaculture*. Calif. Sea Grant College Program, Institute of Marine Resources, Univ. Calif., La Jolla, Calif.: 123–129 & 200–201.
- Shepherd SA, Womersley HBS (1970) The sublittoral ecology of West Island, South Australia. Environmental features and the algal ecology. *Trans. r. Soc. S. Aust.* 94: 105–138.
- Torres M, Niell FX, Algarra P (1991) Photosynthesis of *Gelidium sesquipedale*: effects of temperature and light on pigment concentration, C/N ratio and cell-wall polysaccharides. *Hydrobiologia* 221 (Dev. Hydrobiol. 68): 77–82.
- Yaphe W, Duckworth M (1972) The relationship between structures and biological properties of agars. *Proc. 7th Int. Seaweed Symp.* 7: 15–22.
- Young K, Duckworth M, Yaphe W (1971) The structure of agar. Part III. Pyruvic acid, a common feature of agars from different agarophytes. *Carbohydr. Res.* 16: 446–448.