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Seasonal variation in agar composition and properties from *Gracilaria gracilis* (Gracilariales, Rhodophyta) of the Patagonian coast of Argentina

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SUMMARY

Seasonal variation of agar from specimens of a commercially exploited population of *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine & Farnham in the Patagonian coast of Argentina was studied. For each seasonal harvest, random samples of plants were pooled for subsequent polysaccharide extraction at different water temperatures and agar physico-chemical properties and composition were determined.

Both spring and summer plants yielded 30% and 41% of agar, respectively, which differed slightly in their *at rest* rheological behavior. Spring and summer plants produced strong gels (238 and 218 g cm^{-2}, respectively), but the latter gels had nil adhesiveness. In autumn plants, agar yield decayed to 19%, though the product still maintained similar gel strength (210 g cm^{-2}). Adhesiveness in this product was at least an order of magnitude higher than in the others, concomitant with a peak in the formation of tetraspores and carpospores. This suggests a biological role for the galactan in the initial attachment of spores to the substrate. But since fall corresponds to the settling of reproductive structures, caution should be taken to harvest the algae once spores have been shed from mother plants.

Key words: agar, *Gracilaria gracilis*, rheological behavior, seasonal variation.

INTRODUCTION

The genus *Gracilaria* Greville (Gracilariales, Gracilariaceae, Rhodophyta) includes worldwide species of economical significance (Smit 2004) in the phycocolloid industry. Due to its agar's high yield and quality, *G. gracilis* (Stackhouse) Steentoft, Irvine & Farnham together with *G. chilensis* Bird, McLachlan & Oliveira are the most frequently exploited species (Oliveira *et al.*, 2000). In Argentina, *G. gracilis* grows exclusively in the Chubut Province, where the agar industry is based on collection from the commercial beds located in small bays in the north of Golfo San Jorge, such as Bahía Bustamante, Bahía Melo and Bahía Arredondo. Being the most productive, the population of Bahía Bustamante has been exploited since the end of the 1960s, leading to a notorious decrease in annual production (Boraso *et al.*, 2006). Aiming to achieve a sustainable exploitation of natural beds, a monitoring of the annual cycle of the population was undertaken (Martín *et al.*, 2010) in order to design the appropriate harvest schedule to promote resource renewal and/or propagation. Summer plants produce a good quality agarose with a 71% yield, which does not require alkaline pretreatment, even when extracted at 70°C (Rodríguez *et al.*, 2009).

Composition and properties of *Gracilaria* agars depend on, among others, the species, physiological factors, life cycle stage, environmental conditions including seasonal and geographical aspects, extraction procedures and post-harvest storage (Marinho-Soriano & Bourret 2003; Marinho-Soriano *et al.*, 2006; Romero *et al.*, 2008). Yet, knowledge on seasonal variation in chemical composition and physical properties of agar extracted from *G. gracilis* from Bahía Bustamante has not been informed.

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The aim of the present contribution is to relate the yield, chemical composition and physical properties of agar with environmental factors and reproductive stages along the year in order to contribute to a sustainable commercial exploitation of Bahía Bustamante algal resource.

MATERIALS AND METHODS

Sample collection

Gracilaria gracilis was collected between March 2006 and February 2008 from the subtidal in Bahía Bustamante (45°08′S, 66°32′W), Chubut Province, Argentina. Specimens were collected by SCUBA diving. The sampling units were placed on four transects parallel to the shore laid 100 m from each other and beginning 100 m away from the shore. On each transect five square sampling units (0.25 m^2) were located at 100 m intervals. Each sampling unit was relocated by GPS, being latitude and longitude for the first square sampling unit on each transect the following: 45°08.815′S, 66°29.107′W; 45°08.768′S, 66°29.142′W; 45°08.695′S, 66°29.190′W and 45°08.630′S, 66°29.198′W. For seasonal variation in agar physico-chemical properties, at least 30 random specimens from May 2006 (autumn), August 2006 (winter), October 2006 (spring) and January 2007 (summer) were sampled from inside each of the square sampling units. Specimens corresponding to each season were pooled, air dried and milled for subsequent polysaccharide extraction.

Environmental physico-chemical variables

For each sampling site, *in situ* data of temperature, salinity and pH were taken from the subsurface water. Water samples were also collected for nutrient analysis, keeping them refrigerated until arrival at the laboratory. Nutrient concentrations were analyzed by colorimetric techniques on a Technicon II autoanalyzer (Emeryville, CA, USA), at the Marine Chemistry Laboratory of the Instituto Argentino de Oceanografía (IADO). Nitrates, nitrites and phosphates were determined as indicated in Martín *et al*. (2010).

Polysaccharide extraction procedure

Previously milled random selected plants (10 g dry weight) were mechanically stirred in water (1 L) at room temperature (×3) for 24 h. Room temperature extracts were pooled (extract RTW). Extraction continued in: (i) water (1 L) at 70°C (×3) for 4 h (fractions W70.1–3) and (ii) water (1 L) at 90°C (×3) for 4 h (fractions W90.1–3). After each extraction step, the residue was removed by centrifugation and the supernatant was concentrated and freeze-dried. Extracts obtained at room temperature were thoroughly dialyzed using Spectra/Por tubing (Spectrum Laboratories, Rancho Domínguez, CA, USA) with a molecular weight cutoff of 3500 Da before freeze-drying. Dialyses were performed during 48 h in running tap water followed by 24 h dialysis in a closed system against distilled water.

Composition of the products

Carbohydrate content was analyzed by the phenol-sulfuric acid method (Dubois *et al*., 1956) without previous hydrolysis of the polysaccharide, using galactose as standard. Sulfate was measured with the turbidimetric method of Dodgson and Price (1962) after hydrolysis of the samples with 1 M HCl for 4–5 h at 105–110°C. The content of protein was estimated by the method of Lowry *et al*. (1951).

Monosaccharide composition

Reductive hydrolysis of the polysaccharide samples and further acetylation of the sugar mixtures were performed as described by Stevenson and Furneaux (1991).

Gas Liquid Chromatography (GLC) of alditol acetates was carried out on a Hewlett-Packard 5890A Gas Chromatograph (Hewlett Packard, Avondale, PA, USA) equipped with a flame-ionization detector and fitted with a fused-silica column (0.25 mm i.d. × 30 m) WCOT-coated with 0.20 μm film of SP-2330 (Supelco, Bellefonte, PA, USA). Chromatography of the alditol acetates was carried out isothermally at 220°C. Nitrogen was used as carrier at a flow rate of 1 mL min^{-1}. The split ratio was 80:1. The injector and detector temperature was 240°C. Conversion of GLC areas to molar basis was calculated according to the effective carbon response theory (Sweet *et al*., 1975).

All data are expressed as means of duplicates.

Alkaline treatment and determination of cyclizable α-galactose 6-sulfate units

One-pot alkaline treatment was carried out as described by Navarro and Stortz (2003). The polysaccharide (10 mg) was dissolved in water (5 mL) and $NaBH_4$ (2 mg) was added. After 1 h, 5 M NaOH (1.7 mL) was added and the solution heated at 80°C in a water bath. According to previous results (Rodríguez *et al*., 2009), the cyclization reaction was stopped after 5 h by neutralization with 4 M trifluoroacetic acid. The solvent was evaporated-off and the residue derivatized to the acetylated alditols according to Stevenson and Furneaux (1991).

Gel physical properties

Agar (1.5% w/w in water) was hydrated during 4 h at room temperature in closed flasks with stirring. Afterwards, heating proceeded at 90°C for 30 min until homogeneous viscous solutions were obtained. The hot agar solutions were placed in glass test tubes, cooled and allowed to gel overnight at room temperature to give cylindrical gel specimens (1.6 cm diameter × 10 mm height).

The mechanical studies were carried out in an Instron Universal Testing Machine (Model 3345, Instron, Norwood, MA, USA). Compression tests were performed until fracture under two different rates of compression: 1 mm min^{-1} and 1 mm s^{-1}. The texture profile analysis (TPA) was performed at a rate of compression of 1 mm min^{-1} and a deformation of 20%, in order to allow reproducibility in the results. A cylindrical probe (3.0 cm diameter × 1.0 cm height) was used in all assays. The following parameters were obtained for all the samples according to Rosenthal (1999): (i) gel strength (ratio between the fracture force at compression and the area of the sample, expressed in g cm^{-2}); (ii) the elasticity index (quotient between the time needed to attain the higher force in the second compression and the time necessary for attaining the maximum in the first compression-TPA assay); (iii) resilience (the ratio of the work between the withdrawal and the work during the first compression-TPA assay); and (iv) adhesiveness (negative area calculated from the curve during the first retraction of probe-TPA assay, expressed in g min^{-1}). Elasticity refers to the ability of a gel to regain its original shape once the probe is removed, while resilience is the amount of energy it can store while being compressed without creating a permanent distortion. The adhesiveness is the capacity of the gel to overcome the attractive forces with the substrate it contacts.

Data for analyzed W70.1 samples were expressed as means of duplicates.

Rheological characterization

The W70.1 product (0.0050 g) of autumn, winter, spring and summer harvests was suspended in deionized water and vortexed until complete hydration in order to get a 0.5% w/w final concentration. Then, systems were stored at 25°C for 18 h to attain swelling equilibrium before measurement.

Rheological characterization was accomplished using a RheoStress RS600, controlled stress rheometer (Haake, Karlsruhe, Germany) equipped with a PP35 serrated parallel plate (Haake, Karlsruhe) geometry (35 mm-diameter). A gap size of 500 μm was set, and data points were recorded at steady-state.

Amplitude sweeps were first performed in order to determine the linear viscoelastic range (LVR). Storage (G′) and loss (G″) shear moduli as well as strain were recorded as a function of stress, at a constant frequency of 0.1 Hz and at 25°C in the LVR without affecting gel structure (evaluations *at rest*). The constant stress value to use in the subsequent frequency sweeps was chosen according to the linear viscoelastic range previously determined for each agarose sample. Each mechanical spectrum was then obtained at the selected constant stress value, recording G′, G″, and Tan δ (loss tangent) as a function of increasing angular frequency (ω), after reaching steady state condition for each point.

Determinations were repeated at least twice for each sample.

Transmission electron microscopy

Four season *G. gracilis* samples were fixed at 5°C for 12 h in 0.1 M Na-cacodylate buffer (pH 7.4) containing 0.25 M sucrose, 3% glutaraldehyde and 1.5% paraformaldehyde. Fixation was followed by a series of rinses in the buffer with gradually decreasing concentrations of sucrose. Then, the samples were post-fixed in 2% OsO_4 in the buffer, dehydrated in acetone and infiltrated in Spurr's resin. Thin sections were stained with aqueous uranyl acetate followed by lead citrate and observed in a JEOL 100CX-II transmission electron microscope operated at 80 kV.

RESULTS

The composition of the aqueous extracts RTW, W70.1 and W90.1 obtained from the four season algal material is depicted in Table 1. Due to their low yield, only composition and monosaccharide analyses were performed for products W70.2–3 and W90.2–3 (Table 2).

Regardless of the harvest season, higher agar yield was obtained after the first extraction at 70°C. Four season W70.1 fractions did not differ in their composition or in their sulfation degree (between 4% and 6%). High glucose molar percentages would suggest higher floridean starch content in summer plants. Accordingly, transmission electron microscopy (TEM) observations of summer specimens showed abundant floridean starch granules when compared to autumn ones (Fig. 1). This increment in starch content was concomitant with lower nitrogen content in water (Table 3). On the other hand, higher nitrogen and phosphate content in water coincided with important thylakoidal development in algal cortical cells (Fig. 1a) and higher protein content in the products extracted from autumn-winter plants (Table 1).

The highest gel strength for spring or summer agar was in agreement with high elasticity and resilience indexes for these samples (Table 4). On the contrary,

Table 1. Yields and analyses of the products obtained at room temperature (RTW), 70°C (W70.1) and 90°C (W90.1) from *Gracilaria gracilis* collected in autumn (May 2006), winter (Aug 2006), spring (Oct 2006) and summer (Jan 2007)

Product	Yield (%)†	Carbohydrate (%)	Protein (%)	Sulfate (%)	Monosaccharide composition (mole %) AnGal	6-Me Gal	Xyl	3/4-Me Gal	Gal	Glc
RTW										
May 2006	2	48	20	7	n.d. ‡	n.d.	n.d.	n.d.	n.d.	n.d.
Aug 2006	2	59	16	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Oct 2006	3	57	15	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Jan 2007	3	63	12	9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
W70.1										
May 2006	19	61	9	4	45	7	1	1	41	5
					44§	**8**	**2**	**1**	**42**	**3**
Aug 2006	15	55	9	4	40	9	1	1	41	8
					37	**11**	**2**	**1**	**43**	**6**
Oct 2006	30	74	8	6	41	9	1	2	38	9
					41	**9**	**1**	**2**	**38**	**9**
Jan 2007	41	64	4	4	40	5	1	1	39	14
					41	**6**	**2**	**1**	**38**	**12**
W90.1										
May 2006	8	69	12	6	41	11	2	4	34	8
Aug 2006	7	74	12	6	34	4	0	3	41	18
Oct 2006	6	88	7	6	38	12	1	3	35	11
Jan 2007	3	75	6	5	33	8	3	3	32	21

†Expressed as percentage of lyophilized alga; ‡ n.d., not determined; § Numbers in bold correspond to monosaccharide molar percentages in hydrolyzed product after alkaline treatment.

Table 2. Yields and analyses of the products obtained after second and third aqueous extractions at 70°C (W70.2 and W70.3) and 90°C (W90.2 and W90.3) from *Gracilaria gracilis* collected in autumn (May 2006), winter (Aug 2006), spring (Oct 2006) and summer (Jan 2007)

Product	Yield (%)	Carbohydrate (%)	Protein (%)	Sulfate (%)	Monosaccharide composition (mole %) AnGal	6-Me Gal	Xyl	3/4-Me Gal	Gal	Glc
W70.2										
May 2006	3	40	18	3	31	4	2	0	48	15
Aug 2006	7	24	11	3	34	4	2	0	38	21
Oct 2006	8	55	7	2	20	3	2	0	33	42
Jan 2007	3	61	6	3	28	3	1	0	41	27
W70.3										
May 2006	2	56	14	4	30	6	0	0	48	15
Aug 2006	5	59	15	3	51	8	0	0	34	7
Oct 2006	3	88	9	4	44	7	0	0	37	12
Jan 2007	4	77	9	5	39	4	0	0	36	21
W90.2										
May 2006	3	70	19	5	30	9	3	5	34	19
Aug 2006	5	73	14	5	33	5	1	3	42	17
Oct 2006	1	70	12	5	20	8	3	2	30	37
Jan 2007	2	82	4	4	24	6	8	3	21	38
W90.3										
May 2006	2	68	16	5	31	10	3	5	40	12
Aug 2006	3	62	10	4	22	3	0	3	32	41
Oct 2006	1	61	15	5	27	5	1	3	31	33
Jan 2007	1	74	6	4	14	3	4	2	26	50

W70.1 of winter specimens exhibited notable lower gel strength (Table 4), which could be attributed to differences neither in composition nor in sulfation degree (Table 1). Moreover, alkaline treatment led to no increment in 3,6-anhydrogalactose molar percentage, indicating negligible sulfate substitution on the C-6-position of the α-L-galactose unit. On the other hand, the autumn polysaccharide showed the highest adhesiveness value, while summer samples exhibited the lowest one (value: 0) (Table 4). The highest

Table 3. Physicochemical properties of water, nutrient content and ratio of reproductive to vegetative plants at harvest time in Bahía Bustamante

Harvest time	Salinity (‰)	Temperature (°C)	pH	Nitrites (μmol L^{-1})	Nitrates (μmol L^{-1})	Phosphates (μmol L^{-1})	N:P ratio	Reproductive to vegetative plants†
May 2006	33.6	13	7.4	0.24	5.65	2.18	2.70	49
Aug 2006	33.6	9.5	7.3	0.13	1.56	0.92	1.84	2.4
Oct 2006	33.3	12	7.7	0.06	0.08	1.69	0.08	2.5
Jan 2007	33.7	16.5	7.6	0.07	0.51	1.20	0.48	2.5

†(Gametophytes + tetrasporophytes)/non reproductive plants.

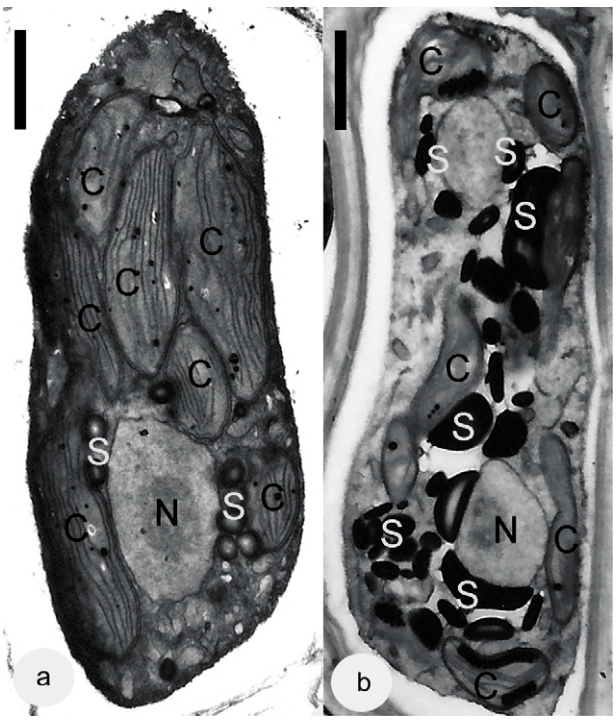

Fig. 1. Transmission electron micrographs of *Gracilaria gracilis* cortical cells. Longitudinal sections perpendicular to the thallus surface. (a) Autumn sample. Note well-developed chloroplasts. (b) Summer sample. Note abundant floridean starch granules. C, chloroplast, S, floridean starch, N, nucleus. Scale bars = 2 μm.

adhesiveness value was concomitant with a noteworthy increase in reproductive phases (gametophytes plus tetrasporophytes) (Table 3).

Data obtained from aqueous systems through oscillatory test at linear condition (*at rest* experiences) indicated that all systems presented G′ (*storage moduli*) > G″ (*loss moduli*) being the difference of one order of magnitude and G′ practically frequency independent (Fig. 2) along the three frequency decades sweep (0.1–100 rad s^{-1}). Summer W70.1 sample exhibited lower values of G′ and G″ and the lowest values of Tan δ, denoting the greatest proportion of elastic behavior. G″ showed, in general, the typical minima of some biopolymer networks at lower frequency values (Doublier *et al.*, 1992; Grassi *et al.*, 1996), especially for W70.1 from

Table 4. Seasonal variation in rheological properties of 1.5% gels of W70.1 from *G. gracilis*

Agar	Gel strength (g cm^{-2})†	Elasticity	Resilience	Adhesiveness (g min^{-1})
May 2006	210	0.83	0.54	3.08
Aug 2006	75	0.92	0.70	0.24
Oct 2006	238	0.95	0.60	0.57
Jan 2007	218	0.92	0.69	0

†Gel strength determined using a rate of compression of 1 mm s^{-1}. For a rate of 1 mm min^{-1} the following values were obtained: 84, 30, 95 and 87 g cm^{-2} for May 2006, Aug 2006, Oct 2006 and Jan 2007, respectively.

winter, spring and summer, which exhibited a certain non-ideality in their gel behavior (Clegg 1995).

DISCUSSION

As in other high latitude algal populations (Kain & Destombe 1995), biomass of *G. gracilis* population in Bahía Bustamante exhibited seasonality in biomass production, oscillating from a minimum in winter to a maximum in late spring and summer (Martín *et al.*, 2010). Rebello *et al.* (1996) stated that temperature influences *G. gracilis* growth, being the optimum approximately 18°C, a value proximate to summer water temperature in Bahía Bustamante.

Higher carbohydrate content would be expected in spring-summer plants due to higher bulk photosynthetic carbon fixation under longer day conditions. Such trend was suggested by the total yield of W70.1 (30 and 41% for spring and summer plants, versus 19% and 15% for autumn and winter plants). Positive relation between biomass production and agar yield has been informed for *G. gracilis* (Rebello *et al.*, 1996; Marinho-Soriano & Bourret 2003), *Gracilaria* sp. (Chirapart & Ohno 1993), *G. verrucosa* (Yenigül 1993) and *G. vermiculophylla* (Vergara-Rodarte *et al.*, 2010). On the other hand, an inverse relation between seawater temperatures and agar yields has been reported by Bird and Ryther (1990) and Mollet *et al.* (1998).

While agar yield declines in rapid growing young tissues, gel strengths have been reported to be higher at

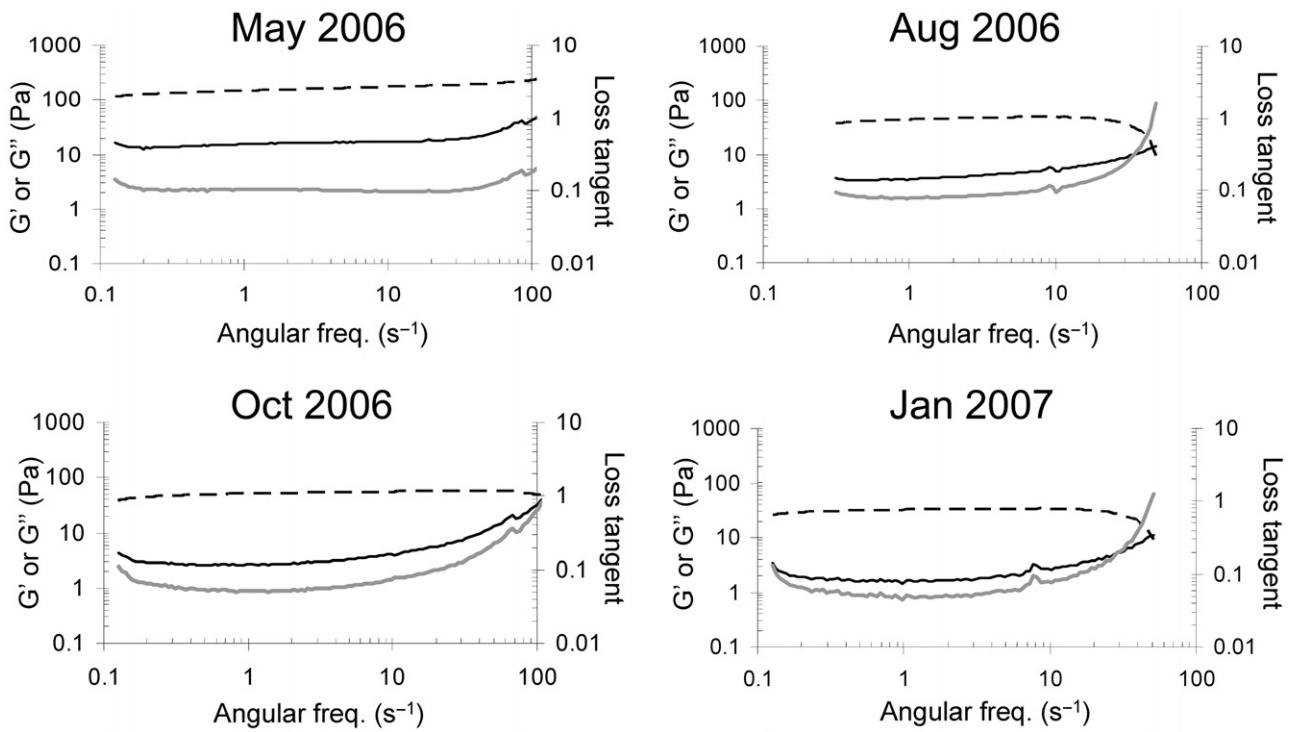

Fig. 2. Mechanical spectra of 0.5% w/w-aqueous systems of agaroses (W70.1) extracted from plants collected in May 2006, Aug 2006, Oct 2006 and Jan 2007 (25°C). Dashed line, storage modulus (G′), black line, loss modulus (G″), grey line, loss tangent (Tan δ).

the time of rapid growth (Craigie & Wen 1984; Chirapart & Ohno 1993). Actively growing plants produce young tissues, which contain an agar with larger proportion of non-substituted α-L-galactose, together with a higher proportion of 3,6-anhydrogalactose, thus yielding stronger gels. The extracted material in the present work was not previously sorted and, thus, consisted of both young, rapidly growing thalli together with mature plants. In such case, high polysaccharide yield and high gel strength were obtained, but it is impossible to determine either the contribution of mature tissues to agar yield or young tissues to gel strength.

Marinho-Soriano and Bourret (2005) studied the gel strength of solutions (1.5% w/v) of agars from *Gracilaria dura* harvested in Thau lagoon (Mediterranean Sea, France) and observed that the higher values were obtained in October (autumn) and the lowest in June (spring-summer) achieving values of 600 g cm^{-2} and 263 g cm^{-2}, respectively. Rebello *et al*. (1997) studied six species of *Gracilaria* from different parts of the world observing different behaviors according to harvesting location and NaOH concentration in extracting solution. Besides features related to the species of the extracted material and/or geographical locations, variability in gel strength values can be ascribed to extraction procedures, diverse experimental conditions and /or concentration of systems tested in each case. Unfortunately, the determination of gel strengths in agars is not regulated by a unique standard procedure as, for instance, in gelatin, which is normalized by the Gelatin Manufacturers Institute of America (AOAC 1986). Additionally, many agarose rheological reports do not adequately inform the conditions used for the determination (i.e. polysaccharide concentration, sample size, crosshead rate) precluding the possibility of comparison with the values herein informed. In our case, remarkable lower gel strength resulted for winter plants, a fact that can be attributed neither to different composition nor sulfation degree. Lower molecular weight or a high polydispersion in molecular weight for winter material could account for lower gel strength.

The presence of floridean starch in the product W70.1 from summer and spring plants can be interpreted as a direct consequence of carbon assimilation. Starch and floridoside tend to increase during nutrient limitation (Collén *et al*., 2004). In fact, lower phosphate and nitrogen water content were dosed in spring and summer. Nevertheless, a decreasing trend in nitrogen does not limit biomass production (Martín *et al*., 2010). This has been explained as a nitrogen storage strategy in this species (Smit *et al*., 1997), which allows assimilation and growth under longer day and higher temperature regimes. Collén *et al*. (2004) stated that both starch and floridoside may function as carbon storage at almost the same degree. Moreover, floridoside pool can be used to fuel agar production in long

day conditions, leading to higher agar yield, as was the case in spring-summer material of *G. gracilis.*

According to Lai *et al*. (1999) and Villanueva *et al*. (2010), starch has a negative effect on gel strength in agarose. Despite the presence of floridean starch in W70.1 from spring and summer plants, gel strength remained the higher and Tan δ, the lower. This suggests that extraction at 70°C rendered an agar of high gel strength, even without the application of amylase treatment. It should be noted that changing temperature to 90°C enriched agar fraction in floridean starch, as indicated by the higher glucose content. This fact had a deleterious effect on agarose gelling capacity, as we have previously reported (Rodríguez *et al*., 2009). In industrial processes, freeze-thawing can partially eliminate starch as it dissolves in the thaw water (Chiovitti *et al*., 2004). Yet, starch elimination is not always thus accomplished (Villanueva *et al*., 2010). It is important to note that for *G. gracilis* from Bahía Bustamante, extraction procedure at 70°C constituted a lower energy consuming process yielding an agar with less floridean starch content and good gelling capacity.

The presence of floridean starch in January 2007 samples may be responsible of the lower value of storage moduli (G′) even when it did not significantly affect gel strength. Mohammed *et al*. (1998) studied the rheology of composite gels formed by gelatinization of (uncrosslinked) waxy maize starch (swelling volume ≈ 12 mL g^{-1}) in agarose solution (0.25% w/w) at 80°C and subsequent gelation of the agarose component by rapid quenching to 5°C. At low starch concentrations (up to 2% w/w), the storage moduli of the samples resembled those of the agarose phase, indicating that the swollen granules were present as a dispersed phase within a continuous biopolymer (agarose) matrix. At higher starch concentration (3–5% w/w), there was a sharp reduction in experimental moduli, their values matching closely those calculated for a bicontinuous network.

Protein content was higher in autumn-winter. One of the first responses to the decrease in irradiance (as expected in autumn-winter) is the increase in antenna pigment content (phycobiliproteins) relative to chlorophyll *a* together with a decrease in growth rate (Carnicas *et al*., 1999). But the modulation of photosystems' size is dependent on nitrogen availability (García-Sánchez *et al*., 1993). Higher protein content in autumn-winter plants was not surprising since most of the cellular protein is related to photosystems. Important thylakoid development was observed in algal samples obtained when high nitrate content was dosed. This could play a double role: on the one hand it improves photon capture in light limiting conditions (autumn-winter) and on the other, pigments might act as accessible nitrogen stores under more favorable environmental conditions for growth (spring-summer). Nitrogen storage has also been verified in *G. gracilis* (Smit *et al*., 1997) allowing endurance for long periods by postponing assimilation when environmental conditions (temperature and day length) turn out more favorable. Due to the abundance of phospholipids in thylakoidal membranes, their development was also favored by abundant phosphate supply.

The autumn polysaccharide differed from the other agars regarding its physical properties, especially adhesiveness. The highest adhesiveness was observed for W70.1 isolated from *G. gracilis* collected in May 2006 and the lowest for the one isolated from *G. gracilis* collected in January 2007 (value: 0). Though chemical composition of the autumn product did not show major differences from the others, populational analyses indicated an increment in reproductive phases (gametophytes plus tetrasporophytes). Since initial attachment of spores to substrate depends on mucilage secretion, the greater adhesiveness of the autumn polysaccharides could be related to their biological role.

We may conclude that both spring and summer plants produce agar in good yield that differed slightly in their rheological behavior. The former produced stronger gels with higher adhesiveness while the latter gave strong gels but with zero adhesiveness. In autumn plants, an agar with good gel strength was produced, though in lower yield. Besides, this product presented high adhesiveness, simultaneously with the massive settling of reproductive structures. Consequently, caution should be taken to harvest the algae once spores have been shed from mother plants.

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REFERENCES

AOAC. 1986. Official Procedure of the Gelatin Manufacturers Institute of America.

Bird, K. T. and Ryther, J. H. 1990. Cultivation of *Gracilaria verrucosa* (Gracilariales, Rhodophyta) strain G-16 for agar. *Hydrobiologia* **204/205**: 347–51.

Boraso, A. L., Ciancia, M. and Cerezo, A. S. 2006. Seaweed resources of Argentina. *In* Critchley, A. T., Ohno, M. and Largo, D. B. (Eds) *World Seaweed Resources – An*

Authoritative Reference System. Electronic version 1.0. Eti Information Services Ltd, Amsterdam, 26 pp. http://www.eti.uva.nl/products/

Carnicas, E., Jiménez, C. and Niell, F. X. 1999. Effects of changes of irradiance on the pigment composition of *Gracilaria tenuistipitata* var. *liui* Zhang et Xia. *J. Photochem. Photobiol. B, Biol.* **50**: 149–58.

Chiovitti, A., McManus, L. J., Kraft, G. T., Bacic, A. and Liao, M. 2004. Extraction and characterization of agar from Australian *Pterocladia lucida*. *J. Appl. Phycol.* **16**: 41–8.

Chirapart, A. and Ohno, M. 1993. Seasonal variation in the physical properties of agar and biomass of *Gracilaria* sp. (*chorda* type) from Tosa Bay, southern Japan. *Hydrobiologia* **260/261**: 541–47.

Clegg, S. M. 1995. Thickening, gels and gelling. *In* Beckett, S. T. (Ed.) *Physico-Chemical Aspects of Food Processing*. Chapman and Hall, Glasgow, pp. 117–41.

Collén, P. N., Camitz, A., Hancock, R. D., Viola, R. and Pedersén, M. 2004. Effect of nutrient deprivation and resupply on metabolites and enzymes related to carbon allocation in *Gracilaria tenuistipitata* (Rhodophyta). *J. Phycol.* **40**: 305–14.

Craigie, J. S. and Wen, Z. C. 1984. Effects of temperature and tissue age on gel strength and composition of agar from *Gracilaria tikvahiae* (Rhodophyceae). *Can. J. Bot.* **62**: 1665–70.

Dodgson, K. S. and Price, R. G. 1962. A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochem. J.* **84**: 106–10.

Doublier, J. L., Launay, B. and Cuvelier, G. 1992. Viscoelastic properties of food gels. *In* Rao, M. A. and Steffe, J. F. (Eds) *Viscoelastic Properties of Foods*. Elsevier Applied Science, New York, pp. 371–434.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric method of determination of sugars and related substances. *Anal. Chem.* **28**: 350–56.

García-Sánchez, M. J., Fernández, J. A. and Niell, F. X. 1993. Biochemical and physiological responses of *Gracilaria tenuistipitata* under two different nitrogen treatments. *Physiol. Plant.* **88**: 631–37.

Grassi, M., Lapasin, R. and Pricl, S. 1996. A study of the rheological behaviour of scleroglucan weak gel systems. *Carbohyd. Polym.* **29**: 169–81.

Kain, J. M. and Destombe, C. 1995. A review of the life history, reproduction and phenology of *Gracilaria*. *J. Appl. Phycol.* **7**: 269–81.

Lai, V. M., Huang, A. L. and Lii, C. Y. 1999. Rheological properties and phase transition of red algal polysaccharide-starch composites. *Food Hydrocoll.* **13**: 409–18.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–75.

Marinho-Soriano, E. and Bourret, E. 2003. Effects of season on the yield and quality of agar from *Gracilaria* species (Gracilariaceae, Rhodophyta). *Bioresour. Technol.* **90**: 329–33.

Marinho-Soriano, E. and Bourret, E. 2005. Polysaccharides from the red seaweed *Gracilaria dura* (Gracilariales, Rhodophyta). *Bioresour. Technol.* **96**: 379–82.

Marinho-Soriano, E., Fonseca, P. C., Carneiro, M. A. and Moreira, W. S. 2006. Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresour. Technol.* **97**: 2402–6.

Martín, L. A., Boraso de Zaixso, A. L. and Leonardi, P. I. 2010. Biomass variation and reproductive phenology of *Gracilaria gracilis* in a Patagonian natural bed (Chubut, Argentina). *J. Appl. Phycol.* **23**: 643–54.

Mohammed, Z. H., Hember, M. W., Richardson, R. K. and Morris, E. R. 1998. Co-gelation of agarose and waxy maize starch. *Carbohydr. Polym.* **36**: 37–48.

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

Navarro, D. A. and Stortz, C. A. 2003. Determination of the configuration of 3,6- anhydrogalactose and cyclizable α-galactose 6-sulfate units in red seaweed galactans. *Carbohydr. Res.* **338**: 211–18.

Oliveira, E. C., Alveal, K. and Anderson, R. 2000. Mariculture of the agar-producing Gracilarioid red algae. *Rev. Fish. Sci.* **8**: 345–78.

Rebello, J., Ohno, M., Critchley, A. T. and Sawamura, M. 1996. Growth rates and agar quality of *Gracilaria gracilis* (Stackhouse) Steentoft from Namibia, Southern Africa. *Bot. Mar.* **39**: 273–79.

Rebello, J., Ohno, M., Ukeda, H. and Sawamura, M. 1997. Agar quality of commercial agarophytes from different geographical origins. 1. Physical and rheological properties. *J. Appl. Phycol.* **8**: 517–21.

Rodríguez, M. C., Matulewicz, M. C., Noseda, M. D., Ducatti, D. R. and Leonardi, P. I. 2009. Agar from *Gracilaria gracilis* (Gracilariales, Rhodophyta) of the Patagonic coast of Argentina. Content, structure and physical properties. *Bioresour. Technol.* **100**: 1435–41.

Romero, J. B., Villanueva, R. D. and Montaño, M. N. 2008. Stability of agar in the seaweed *Gracilaria eucheumatoides* (Gracilariales, Rhodophyta) during postharvest storage. *Bioresour. Technol.* **99**: 8151–55.

Rosenthal, A. J. 1999. *Food Texture: Measurement and Perception*. Aspen Publishers Inc., Gaithersburg, MA.

Smit, A. J. 2004. Medicinal and pharmaceutical uses of seaweed natural products: A review. *J. Appl. Phycol.* **16**: 245–62.

Smit, A. J., Robertson, B. L. and Preez, D. R. 1997. Influence of ammonium-N pulse concentrations and frequency, tank condition and nitrogen starvation on growth rate and biochemical composition of *Gracilaria gracilis*. *J. Appl. Phycol.* **8**: 473–81.

Stevenson, T. T. and Furneaux, R. H. 1991. Chemical methods for the analysis of sulphated galactans from red algae. *Carbohydr. Res.* **210**: 277–98.

Sweet, D. P., Shapiro, R. H. and Albersheim, P. 1975. Quantitative analysis by various glc response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydr. Res.* **40**: 217–25.

Vergara-Rodarte, M. A., Hernández-Carmona, G., Rodríguez-Montesinos, Y. E., Arvizu-Higuera, D. L., Riosmena-Rodríguez, R. and Murillo-Álvarez, J. I. 2010. Seasonal variation of biomass and agar from *Gracilaria vermiculophylla* and the effect of alkali treatment time, stability of its Colagar. *J. Appl. Phycol.* **22**: 753–59.

Villanueva, R. D., Romero, J. B., Ragasa, A. L. and Montaño, M. N. 2010. Agar from the red seaweed *Laurencia flexilis* (Ceramiales, Rhodophyta) from northern Philippines. *Phycol. Res.* **58**: 151–56.

Yenigül, M. 1993. Seasonal changes in the chemical and gelling characteristics of agar from *Gracilaria verruscosa* collected in Turkey. *Hydrobiologia* **260/261**: 627–31.