Hidrobiológica 2021, 31 (2): 125-135

UNIVERSIDAD AUTÓNOMA METROPOLITANA
Casa abierta al tiempo
Unidad Iztapalapa

HIDROBIOLÓGICA
http:/hidrobiologica.izt.uam.mx
ISSN: 2448-7333

OPEN ACCESS
Research Article
August, 2021

Spatial and seasonal effects on physicochemical properties of native agar from *Gracilaria parvispora* (Rhodophyta) in the Tropical Mexican Pacific (Oaxaca-Chiapas)

Efecto espaciotemporal en las propiedades fisicoquímicas del agar nativo de *Gracilaria parvispora* (Rhodophyta) en el Pacífico Mexicano Tropical (Oaxaca-Chiapas)

María de Jesús Trejo-Méndez[1], Gustavo Hernández-Carmona[2], Dora Luz Arvizu-Higuera[2], Edgar Francisco Rosas-Alquicira[1], José Alberto Montoya-Márquez[1], Yoloxochitl Elizabeth Rodríguez-Montesinos[2] and Mauricio Muñoz-Ochoa[2]

Recibido: 04 de julio de 2019. ***Aceptado:*** 09 de julio de 2021. ***Publicado:*** agosto de 2021.

[1] Instituto de Recursos, Universidad del Mar. Ciudad Universitaria s/n, San Pedro Pochutla, Oaxaca, 70902. México

[2] Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional. Av. Instituto Politécnico Nacional s/n, col. Playa Palo de Santa Rita, La Paz, B.C.S., 23096. México

***Corresponding author:**
Edgar Francisco Rosas-Alquicira: e-mail: erosas@angel.umar.mx.

To quote as:
Trejo-Méndez, M. de J., G. Hernández-Carmona, D. L. Arvizu-Higuera, E. F. Rosas-Alquicira, J. A. Montoya-Márquez, Y. E. Rodríguez-Montesinos & M. Muñoz-Ochoa. 2021. Spatial and seasonal effects on physicochemical properties of native agar from *Gracilaria parvispora* (Rhodophyta) in the Tropical Mexican Pacific (Oaxaca-Chiapas). *Hidrobiológica* 31 (2): 125-135.

DOI:10.24275/uam/izt/dcbs/hidro/2021v31n2/Trejo

ABSTRACT

Background: *Gracilaria parvispora* is an invasive red seaweed located in coastal lagoons along the Tropical Mexican Pacific. *Gracilaria* species are the main source of agar around the world. **Goals:** Spatial and seasonal trends of the properties of native agar from the invasive seaweed *G. parvispora* were determined in three localities in the states of Oaxaca and Chiapas belonging to coastal lagoons along the Tropical Mexican Pacific: Ballenato, Paredón, and San Vicente. **Methods:** Native agar was obtained from dry samples of seaweed and the agar yield, gel strength, melting and gelling temperatures, hysteresis, and sulfate and 3,6-anhydrogalactose content were determined for each sample. Moreover, the polysaccharide structures and the location of sulfate groups in agar samples were identified. **Results:** The phycocolloid is a polysaccharide agar type. The agar yield was significantly different between seasons and localities, with the highest values during the dry season (19.9 ± 0.004 %) at Paredón (20.6 ± 0.01 %). Gel strength, melting temperature and gel hysteresis showed significant spatial differences; the highest values were obtained in Ballenato (367.3 ± 14.2 g cm^{-2}, 80.2 ± 1.4 °C, 44.3 ± 2.2 °C, respectively); gelling temperature did not show significant differences between localities or seasons. Chemical properties were significantly different between seasons: 3,6-anhydrogalactose content was higher during the dry season (36.2 ± 0.2 %), and sulfate content was higher during the rainy season (12.69 ± 0.21 %). Salinity was significantly different between seasons, and the highest was obtained during the dry season (38.7 ± 0.1). Surface water temperature varied between localities, and the highest mean value was recorded at Paredón (32.5 ± 0.2 °C). **Conclusions:** The chemical properties of the *G. parvispora* native agar were lower than the standards for food and industrial use.

Key words: 3,6-Anhydrogalactose, coastal lagoons, FTIR-ATR spectroscopy, seasonal variation, sulfate content.

RESUMEN

Antecedentes: *Gracilaria parvispora* es un alga roja invasora que se encuentra en lagunas costeras del Pacífico Mexicano Tropical. A nivel mundial, las especies de *Gracilaria* son la fuente principal de agar. **Objetivos:** Se determinaron las tendencias espaciales y temporales de las propiedades del agar nativo de *G. parvispora* en tres localidades de los estados de Oaxaca y Chiapas, pertenecientes a lagunas costeras del Pacífico Mexicano Tropical: Ballenato, Paredón y San Vicente. **Métodos:** Se determinó el rendimiento del agar, la fuerza de gel, la temperatura de fusión y gelificación, la histéresis, y el contenido de sulfatos y 3,6-anhidrogalactosa del agar nativo obtenido de muestras secas de macroalga. Además, se identificaron las estructuras de los polisacáridos y la posición de los grupos sulfato en las muestras. **Resultados:** El ficocoloide es un polisacárido tipo agar. El rendimiento mostró diferencias significativas entre localidades y temporadas, con valores mayores durante la temporada de secas (19.9 ± 0.004 %) y en Paredón (20.6 ± 0.01 %). La fuerza de gel, la temperatura de fusión y la histéresis mostraron diferencias espaciales significativas; con valores más altos para las muestras de Ballenato (367.3 ± 14.2 g cm^{-2}, 80.2 ± 1.4 °C, 44.3 ± 2.2 °C, respectivamente); la temperatura de gelificación no mostró diferencias significativas entre localidades o temporadas. Las propiedades químicas mostraron diferencias significativas entre temporadas: el contenido de 3,6-anhidrogalactosa fue mayor durante la temporada de secas (36.2 ± 0.2 %), y el contenido de sulfato fue mayor durante la temporada de lluvias (12.69 ± 0.21 %). La salinidad fue significativamente diferente entre temporadas, con mayor valor para la temporada de secas (38.7 ± 0.1). La temperatura superficial del agua varió entre localidades, la más alta se registró en Paredón (32.5 ± 0.2 °C). **Conclusiones:** Las propiedades químicas de *G. parvispora* fueron menores a los estándares para su uso industrial y alimenticio.

Palabras clave: 3,6-anhidrogalactosa, contenido de sulfatos, espectroscopia FTIR-ATR, lagunas costeras, variación estacional.

INTRODUCTION

Agar is one of the three main commercial seaweed extracts, and the most expensive in the hydrocolloids market with a price ranging from 35 to 45 USD kg^{-1} (Callaway, 2015). The world agar industry is based on the red algae *Gelidium*, *Gelidiella*, *Gracilaria* and *Pterocladia* (Armisén & Galatas, 1987; McHugh, 2003); nevertheless, the main agar source worldwide (65%) is extracted from *Gracilaria* species (McHugh, 2002).

Ten species of *Gracilaria* have been recorded from the Tropical Mexican Pacific (TMP) but studies on these species are mainly floristic assessments (e.g., Mendoza-González & Mateo-Cid, 1999; León-Tejera & González-González, 2000; Mateo-Cid & Mendoza-González, 2012). In the coastal lagoons along the TMP, the invasive species *Gracilaria parvispora* I. A. Abbott has been recorded with abundant biomass (Dreckmann, 1999); however, reports about the physical and/or chemical properties of its native agar for the area or another Tropical region are non-existent.

Agar is a complex water-soluble polysaccharide composed of repeating agarobiose units of alternating 1,3-linked D-galactose and 1,4-linked 3,6-anhydro-L-galactose residues (Lahaye & Yaphe, 1988). In order to identify the polysaccharide structures and the location of sulfate groups in agar samples, the infrared (IR) spectroscopy technique has been widely applied. In particular, Fourier-transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) is a technique that provides information from a few milligrams of dried and ground algal material (Murano, 1995; Freile-Pelegrín & Murano, 2005; Gómez-Ordóñez & Rupérez, 2011).

In general, the chemical and physical properties of agar vary with the species and with environmental factors such as light, nutrients, salinity, and temperature (Oyieke, 1993; Armisén, 1995). Vergara-Rodarte *et al.* (2010) found that native agar properties of *G. parvispora* (as *G. vermiculophylla*), inhabiting a Temperate-Tropical region showed seasonal variations, where the highest values for agar yield, gel strength, melting temperature and gelling temperature occurred in summer. In that research, the agar properties were improved by an alkali treatment (Vergara-Rodarte *et al.*, 2010).

In particular, for *Gracilaria* species distributed in Tropical regions, the agar yield and gel strength are higher during the dry season in comparison with the rainy season, and this is associated with high salinity and high seawater temperature (John & Asare, 1975; Marinho-Soriano *et al.*, 2001; Buriyo & Kivaisi, 2003; Orduña-Rojas *et al.*, 2008). Also, higher 3,6-anhydrogalactose (3,6-AG) and sulfate contents occurs in dry season with positive correlations to sea surface water temperature; as well as a negative correlation between 3,6-AG and sulfate content (Oyieke, 1993; Buriyo & Kivaisi, 2003; Freile-Pelegrín & Murano, 2005; Orduña-Rojas *et al.*, 2008).

The aim of this study was to determine spatial and seasonal trends in the physical and chemical properties of *G. parvispora* native agar, from three coastal lagoons located in the TMP, and their correlation with salinity and temperature.

MATERIAL AND METHODS

Study areas. *G. parvispora* was collected in three points located in three localities in the states of Oaxaca and Chiapas: Ballenato in Chantuto-Panzacola Lagoon, Chiapas (August 5, 2015 and April 13, 2016) (15.1661° N, 92.8596° W to 15.1652° N, 92.8609° W); Paredón in Mar Muerto Lagoon, Oaxaca-Chiapas (August 6, 2015 and April 14, 2016) (16.0405° N, 93.8712° W to 16.0523° N, 93.8713° W) ; and San Vicente, in the Superior Lagoon, Oaxaca (August 7, 2015 and April 15, 2016) (16.3692° N, 94.9597° W to 16.3675° N, 94.9598° W). In the region, the rainy season occurs from May to November, and the dry season occurs from December to April (Contreras *et al.*, 1997; Tapia-García *et al.*, 2011).

Sampling. *G. parvispora* thalli were collected along three transects (100 m × 5 m) from August 5-7, 2015 (rainy season) and April 13-15, 2016 (dry season). The collect was carried out only once for each sampling site per season. Transects were placed parallel to the sandy bar, separated from each other by 30 m, and at a maximum depth of 1.5 m. At six points along each transect, environmental parameters (temperature and salinity only) were recorded using a CTD profiler (CastAway™-CTD). The collected seaweeds were sun-dried on the beach and later transported to the laboratory, where they were ground to 0.8 mm and stored in plastic bags at room temperature. Species identification was carried out considering morphological and reproductive characteristics (Abbott, 1985; Dreckmann, 1999; García-Rodríguez *et al.*, 2013).

Agar extraction. The extraction was carried out in triplicate and according to the method of Arvizu-Higuera *et al.* (2008). Fifteen grams of dry seaweed were placed in 800 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 seaweeds were boiled for 1.5 h, and the extract obtained was mixed with diatomaceous earth and filtered under vacuum pressure. The clarified filtrate was allowed to gel at room temperature on plastic trays and then frozen overnight and subsequently thawed at room temperature. The agar was dehydrated with 96% ethanol and dried in an oven at 55°C for 24 h. The agar yield was calculated as the percentage of dry matter.

Physical properties. In order to measure the gel strength, melting and gelling temperatures, a 1.5% solution (w/v) of each native agar sample was prepared. The solution was stored in plastic containers (8 × 3.5 × 3 cm) and allowed to gel at room temperature (22°C) overnight. Gel strength was measured using a texture analyzer (TA.XT plus, Stable Micro Systems, Godalming, Surrey, UK) and Exponent 32® software (Camacho & Hernández-Carmona, 2012). To measure melting temperature, agar solution was poured into a test tube (1.7 cm diameter, 15 cm height) and allowed to gel. Later on, an iron ball (7 mm diameter) was placed on the gel surface. The test tube was clamped in a water bath, and the temperature gradually increased. The melting temperature was recorded with a thermocouple thermometer (Digi-Sense 8528-20, Cole-Parmer Instrument Co., Niles, IL, USA) when the gel started to melt, and the ball sank into the solution. Gelling temperature was measured using the same solution that was allowed to cool down while continuously moving the tube vertically and horizontally. The probe of the thermocouple thermometer was inserted into the solution, and when it ceased flowing, the gelling temperature was recorded. Hysteresis was calculated as the difference between the melting and gelling temperatures (Arvizu-Higuera *et al.*, 2008).

Chemical properties. The 3,6-AG content was determined by the resorcinol-acetal method (Yaphe & Arsenault, 1965), using D-fructose as standard. Absorbance was measured at 555 nm. The concentration of sulfates was determined by the rhodizonate method (Terho & Hartiala, 1971), using sodium sulfate as standard. Absorbance was measured at 520 nm.

Statistical analysis. Data were computed to obtain the average and standard error (±1 SE). Comparisons of physical and chemical properties of native agar samples, both between localities and seasons, were tested with a two-way multivariate analysis of variance (MANOVA), at $\alpha = 0.05$. MANOVA assumptions were evaluated through a residual analysis for the linear model test (Dobson, 2002). When significant differences in the MANOVA test were obtained ($p < 0.05$), a posteriori ANOVA on the significant variables was carried out, and a Tukey test was applied to evaluate levels, factors, and interactions effects (Hair *et al.*, 1999). Also, the Parsons coefficient was computed between agar properties and salinity and temperature values, as well as between agar properties (Statistica v.8 Software, StatSoft 2008). For the variables measured as percentages, in order to accomplish normality assumptions, they were arcsine-transformed (Zar, 2010).

FTIR-ATR spectroscopy analysis. FTIR spectra were acquired with a Perkin Elmer spectrophotometer model TWO with ATR. Each spectrum was obtained by adding 18 scans at a resolution of 4 cm^{-1} in the spectral range of 500-4000 cm^{-1}. The spectra were made with Spectragryph v1.2.10 software (Menges, 2020).

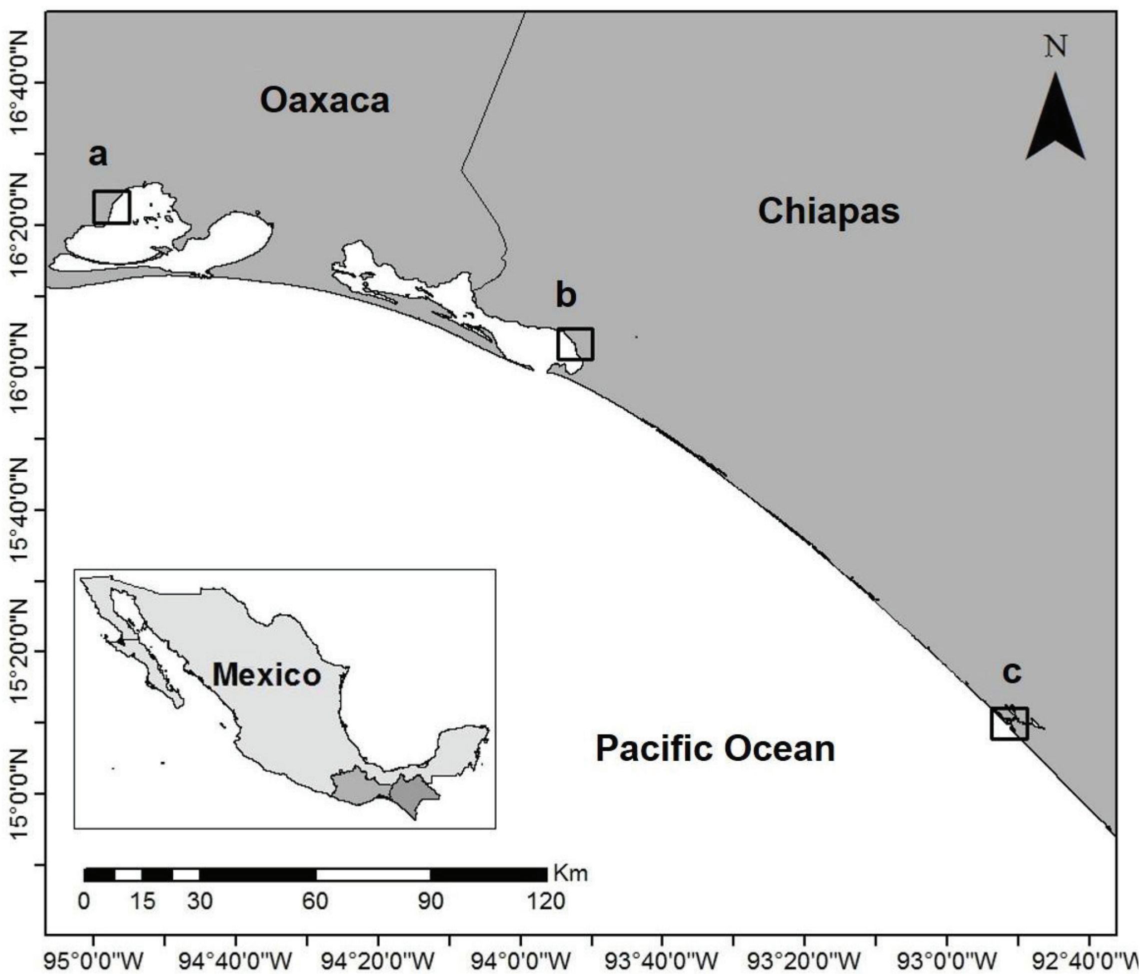

Figure 1. Study area. Sampling sites in coastal lagoons in the Tropical Mexican Pacific: a) San Vicente, b) Paredón, and c) Ballenato.

RESULTS

Agar. Significant differences ($p < 0.05$) were obtained for all the assessed variables (Table 1), except for the gelling temperature that was not affected by the locality, season, or locality/season interaction effect ($p > 0.05$). The significant differences were associated with locality and/or season or their interaction. The locality factor affected agar yield, gel strength, melting temperature and gel hysteresis (Table 2). The season factor was significant for 3,6-AG and sulfate concentration and agar yield. The interaction effect was obtained for agar yield, gel strength, melting temperature and hysteresis (Table 2).

The agar yield during the study period was 18.5 ± 0.01%. The highest agar yield was recorded from Paredón (20.6 ± 0.01%), and it was significantly different from the other localities; also, a significantly higher value was obtained in the dry season (19.9 ± 0.004%) in comparison with the rainy season (17 ± 0.004%) (Fig. 2). For the interaction effect, the agar yield values for Ballenato (19.8 ± 0.01%) and Paredón (23.7 ± 0.01%) during the dry season were significantly higher, while no significant differences were detected between seasons for San Vicente (Fig. 2).

Table 1. Two-way MANOVA of physical and chemical properties of native agar extracted from Gracilaria parvispora, and for the measured environmental parameters.

Effect	λ Wilks	F	DF effect	DF error	p
Physical and chemical agar properties					
Locality	0.024	6.370	12	14	< 0.001*
Season	0.053	20.667	6	7	< 0.001*
Locality/season interaction	0.040	4.694	12	14	0.003*
Environmental parameters					
Locality	0.001	153.77	4	22	< 0.001*
Season	0.011	472.847	2	11	< 0.001*
Locality/season interaction	0.020	35.7	4	22	< 0.001*

DF = Degrees of freedom; F = Proportion of variance; p = Probability values; *significant value ($p < 0.05$).

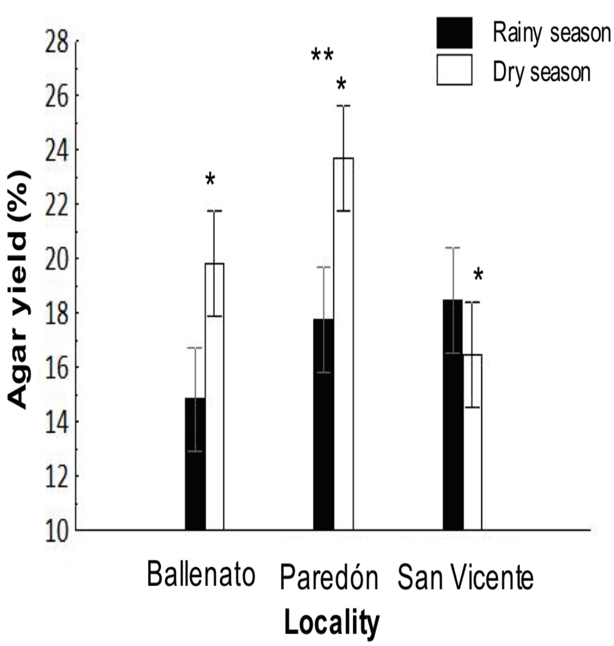

Figure 2. Interaction, locality, or season effects on native agar yield obtained from Gracilaria parvispora collected in three localities in the Tropical Mexican Pacific, n = 3. The asterisk (*) over the dry season bar indicates the presence of single seasonal effect, the agar yield was significantly higher for dry season. Two asterisks (**) indicate the presence of single locality effect, and they are placed over the bars corresponding to the locality that was significantly different.

The values obtained for gel strength, melting temperature, and hysteresis for the study period were 259.2 ± 20.1 g cm^{-2}, 74.6 ± 2.0°C, and 38.1 ± 3.1°C, respectively. The gel strength (367.3 ± 14.2 g cm^{-2}) and melting temperature (80.2 ± 1.4°C) for Ballenato were significantly different to those for the other two localities. A significant difference for hysteresis was found only between Ballenato (44.3 ± 2.2°C) and San Vicente (33.5 ± 2.2°C). Gel strength, melting temperature and hysteresis of agar gels from the Ballenato sample showed higher values (Fig. 3 a-c). Regarding the locality/season interaction effect, for Paredón during the rainy season, gel strength (267.3 ± 20.1 g cm^{-2}), melting temperature (78.5 ± 2.0°C) and hysteresis (43.5 ± 3.1°C) were significantly higher, while for San Vicente during the dry season, melting temperature (79.7 ± 2.0°C) and hysteresis (43.3 ± 2.0°C) were also significantly higher. For Ballenato, no significant difference was detected between seasons (Fig. 3 a-c).

The gelling temperature did not show significant differences between seasons and/or localities or interaction effects. For the study period, the gelling temperature was 35.6 ± 3.9°C; the higher values were obtained for San Vicente during the rainy season, while the lower values were obtained for Paredón during the dry season (Fig. 4).

The 3,6-AG and sulfate content in agar samples obtained in the study period was 33.5 ± 4.5%, and 8.9 ± 4.7%, respectively. The 3,6-AG content was higher in the dry season (36.2 ± 0.02%) than in the rainy season (30.7 ± 0.02%) (Fig. 5a), while the sulfate content was significantly higher in the rainy season (12.69 ± 0.21%) than in the dry season (4.75 ± 0.15%) (Fig. 5b).

Table 2. A posteriori test of physical and chemical properties of native agar extracted from Gracilaria parvispora, and of the measured environmental parameters.

Source of variation	DF	SS	MS	F	P
Agar yield					
Season	1	20.67	20.67	16.139	0.001*
Locality	2	23.68	11.84	9.247	0.003*
Interaction	2	30.37	15.19	11.858	0.001*
Error	12	15.37	1.28		
Gel strength					
Season	1	1874	1874	1.549	0.237
Locality	2	109162	54581	45.112	0.000*
Interaction	2	44504	22252	18.391	0.000*
Error	12	14519	1210		
Melting temperature					
Season	1	9	9	0.718	0.413
Locality	2	285.6	142.8	11.360	0.001*
Interaction	2	665.2	332.6	26.460	0.000*
Error	12	150.8	12.6		
Gelling temperature					
Season	1	8.24	8.24	0.743	0.405
Locality	2	67.28	33.64	3.036	0.086
Interaction	2	47.09	23.54	2.125	0.162
Error	12	132.98	11.08		
Hysteresis					
Season	1	34.51	34.51	1.209	0.293
Locality	2	370.78	185.39	6.495	0.012*
Interaction	2	859.43	429.72	15.055	0.000*
Error	12	342.51	28.54		
3,6-AG					
Season	1	50.34	50.34	10.914	0.006*
Locality	2	1.32	0.66	0.143	0.868
Interaction	2	22.5	11.28	2.445	0.129
Error	12	55.35	4.61		
Sulfates					
Season	1	308.62	308.62	49.808	0.000*
Locality	2	34.36	17.178	2.772	0.102
Interaction	2	30.47	15.238	2.459	0.127
Error	12	74.354	6.196		
Salinity					
Season	1	73.91	73.91	1016.036	0.000*
Locality	2	78.55	39.27	539.926	0.000*
Interaction	2	33.82	16.91	232.472	0.000*
Error	12	0.87	0.07		
Surface water temperature					
Season	1	0.39	0.39	2.399	0.147
Locality	2	22.80	11.40	70.725	0.000*
Interaction	2	12.99	6.49	40.285	0.000*
Error	12	1.93	0.16		

DF = Degrees of freedom; SS = Sum of squares; MS = Mean squares; F = Proportion of variance; p = Probability values; *significant value ($p < 0.05$).

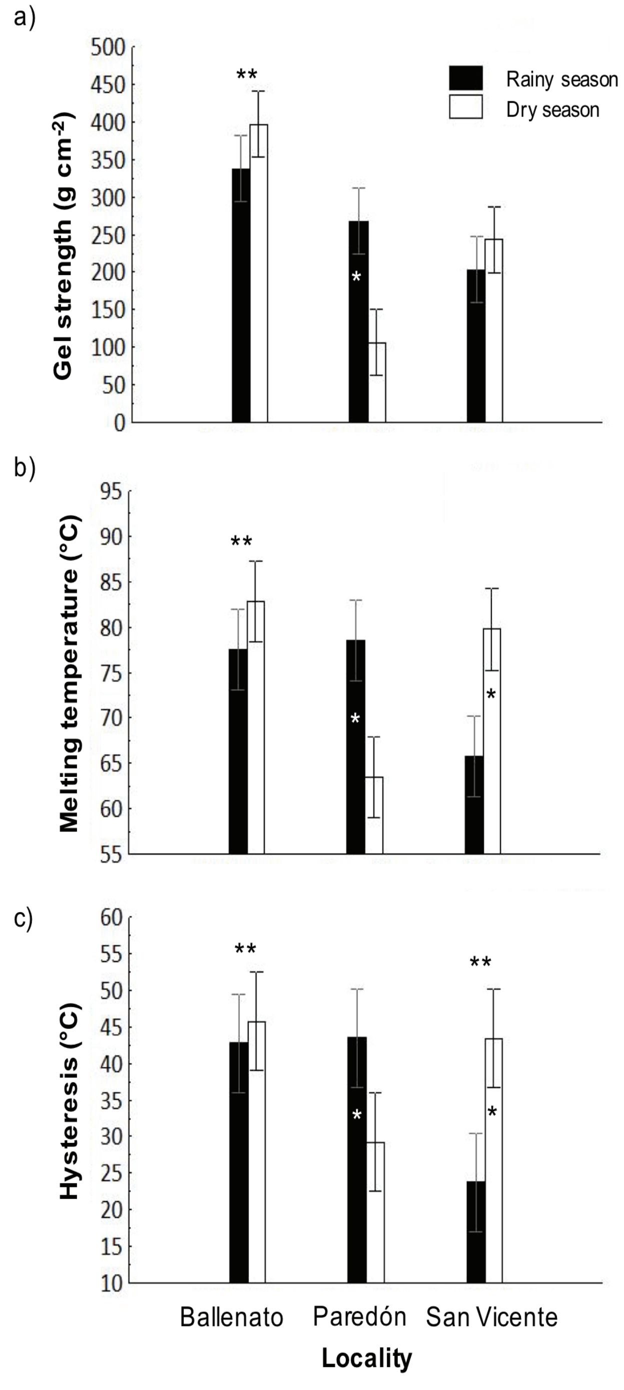

Figure 3 a-c. Interaction, locality, or season effects on gel strength (a), melting temperature (b) and hysteresis (c) of native agar obtained from Gracilaria parvispora collected in three localities in the Tropical Mexican Pacific, n = 3. One asterisk (*) indicates the presence of locality/season interaction effect, the asterisk is placed over the bar season corresponding to the highest values obtained for each locality. Two asterisks (**) indicate the presence of single locality effect, they are placed over the locality bars that presented significant differences. For hysteresis, Ballenato and San Vicente samples were significantly different each other.

Agar properties were correlated with each other in different ways. For example, agar yield showed a negative correlation with gel strength and melting temperature, but a positive correlation with 3,6-AG content. Moreover, gel strength was positively correlated with melting temperature and hysteresis. Melting temperature showed a positive correlation with hysteresis. Finally, hysteresis was negatively correlated with gelling temperature, while sulfate content was negatively correlated with 3,6-AG content (Table 3).

Environmental parameters. Average salinity values were significantly different ($p < 0.05$) among the effects given by locality, season, and for the locality/season interaction (Tables 1 and 2). For the locality effect, the highest salinity was recorded in San Vicente (39.5 ± 0.1), followed by Paredón (32.5 ± 0.1) and Ballenato (30.8 ± 0.1), while a seasonal effect was significant, with higher values during the dry season (38.7 ± 0.1) than during the rainy season (34.6 ± 0.1) (Fig. 6). The salinity was significantly ($p < 0.05$) different for all the localities between seasons.

The surface water temperature was significantly different ($p < 0.05$) between localities and for the locality/season interaction effects (Tables 1 and 2). The highest surface water temperature was in Paredón (32.5 ± 0.2°C), followed by Ballenato (30.8 ± 0.2°C) and San Vicente (29.8 ± 0.2°C), while significantly different values were obtained for all the localities between seasons (Fig. 7).

The relation between salinity and surface water temperature with agar properties showed a negative correlation between gelling temperature and surface water temperature ($r = -0.55$), and a negative correlation between sulfate content and salinity ($r = -0.49$) (Table 3).

FTIR-ATR spectroscopy analysis. The FTIR-ATR spectrum of native agar samples extracted from *G. parvispora* (Figs. 8 and 9) showed a pattern of absorption bands at 1240-1260, 1040, 930, 890, 868, 850 and 820-805 cm^{-1} which correspond to a sulfated polysaccharide agar type (Matsuhiro, 1996; Pereira *et al.*, 2013).

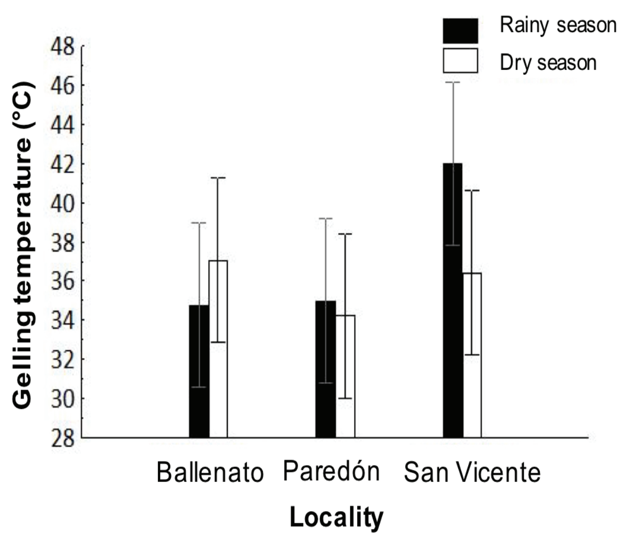

Figure 4. Spatial and seasonal variations in gelling temperature of native agar obtained from Gracilaria parvispora collected in three localities in the Tropical Mexican Pacific, n = 3. No significant differences were detected.

Table 3. Correlations between physical and chemical properties of extracted agar from *Gracilaria parvispora*, and those *versus* environmental parameters.

Agar properties and environmental parameters	Y	GS	MT	GT	H	3,6AG	SO_4C	S	SWT
Agar yield: Y	-	–0.52*	–0.48*	0.06	–0.42	0.48*	–0.10	–0.01	0.00
Gel strength: GS		-	0.79*	0.04	0.64*	0.09	–0.31	–0.25	–0.11
Melting temperature: MT			-	–0.23	0.92*	–0.00	–0.37	0.14	0.17
Gelling temperature: GT				-	–0.59*	0.21	0.27	0.02	–0.51*
Hysteresis: H					-	–0.09	–0.42	0.10	0.34
3,6-AG content: 3,6AG						-	–0.61*	0.21	–0.21
Sulfate content: SO_4C							-	–0.49*	0.07
Salinity: S								-	–0.10
									-

*Significant correlations ($p < 0.05$).

DISCUSSION

This research is the first one about the physical and chemical properties of native agar obtained from *G. parvispora* inhabiting Tropical regions. Overall, the agar properties had significant differences among locations and seasons. There were no significant correlations between agar properties and environmental parameters, except for the gelling temperature vs surface water temperature, and sulfate content vs salinity (Table 3). The lack of correlations could be associated with the prevalence of high salinity and temperature values on the sampling periods, where variations were not biologically significant.

According to the environmental characteristics for the region, the samplings were done at the end of the dry season (April) and in the middle of the rainy season (August) (rainy season from May to November, and the dry season from December to April, Contreras *et al.*, 1997; Tapia-García *et al.*, 2011). Thus, the obtained results in this study are a guide to know how the agar properties are for *G. parvispora* inhabiting in Tropical Mexican Pacific concerning the seasons and localities; however, a continuous sampling would support a complete understanding of the seasonal variation of agar properties.

For *G. parvispora* but inhabiting a Temperate-Tropical region, Vergara-Rodarte *et al.* (2010) determined seasonal effects on yield, gelling temperature, gel strength, and melting temperature in native and alkali-treated agar. Their described patterns agree with the obtained results in the present study, except for gelling temperature. Regarding the obtained values for native agar properties, the results by Vergara-Rodarte *et al.* (2010) are also similar to those of the present study, except for agar yield and gel strength that were higher and lower than those we have obtained, respectively.

In particular, the mean agar yield obtained were 18.5 ± 3.1 %, with a range from 14.8 ± 0.01 % for Ballenato in rainy season to 23.7 ± 0.01 % for Paredón in dry season, respectively. This range was near to those of other invasive species of the Gracilarieae tribe (Gurgel *et al.*, 2018) in Temperate-Tropical regions, e.g., *G. parvispora* (15-34.6%) (Arvizu-Higuera *et al.*, 2008; Krueger-Hadfield *et al.*, 2016, as *G. vermiculophylla*),

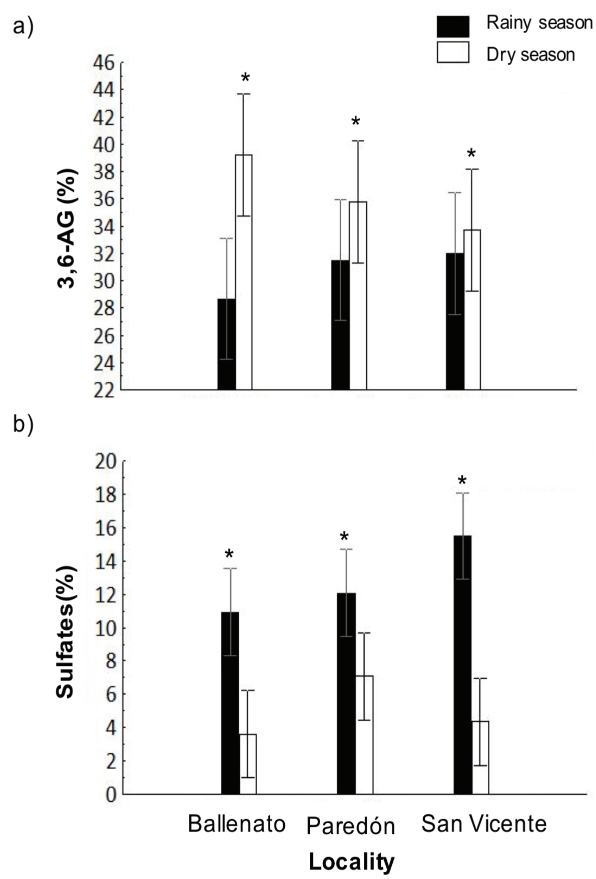

Figure 5 a-b. Interaction, locality, or season effects on 3,6-AG (a) and sulfate content (b) of native agar obtained from Gracilaria parvispora collected in three localities in the Tropical Mexican Pacific, n = 3. Asterisk (*) indicates the presence of single season effects, the asterisk is placed over the season bar corresponding to the highest obtained values for agar properties.

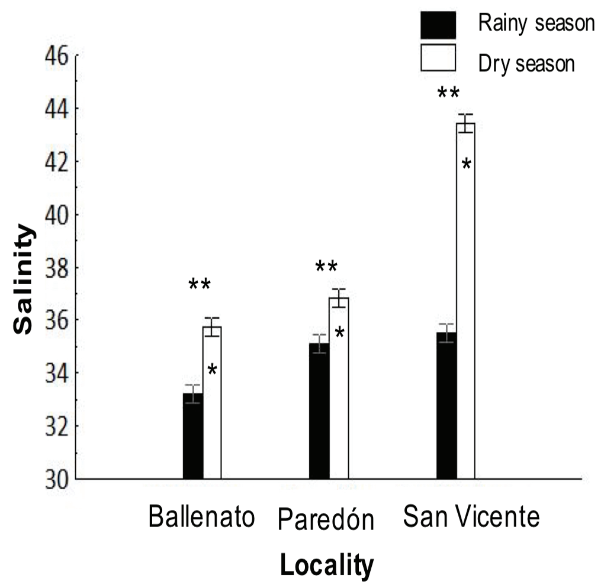

Figure 6. Interaction, locality, or season effects on salinity from the sampled locations, and during the rainy (August 2015) and dry seasons (April 2016). One asterisk (*) indicates the presence of single seasonal effect, and single asterisks are placed over the de bars season corresponding to the highest values obtained for salinity. Two asterisks (**) indicate the presence of single locality effect, and these are placed over the localities bars that presented significant differences between each other for salinity.

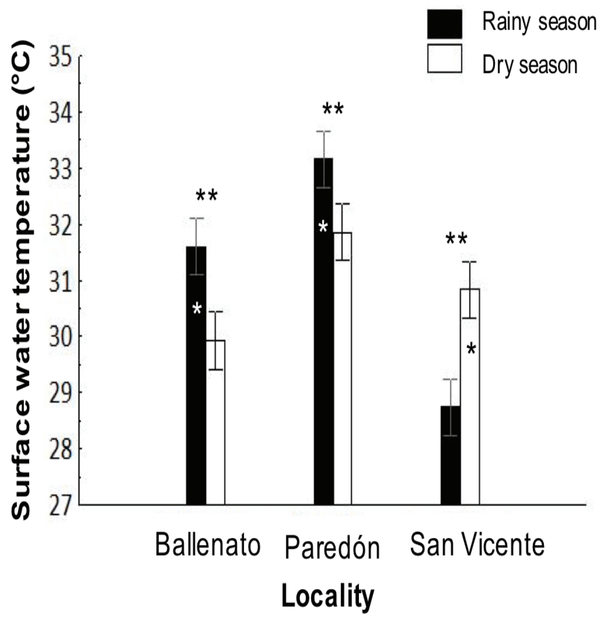

Figure 7. Interaction, locality, or season effects on the surface water temperature from sampled locations, and during rainy (August 2015) and dry seasons (April 2016). One asterisk (*) indicates the presence of single seasonal effect, the asterisk is placed over the bar season corresponding to the highest values obtained for temperature for each locality. Two asterisks (**) indicate the presence of single locality effect, and they are placed over the localities bar with significant differences. All the temperatures were significant different among localities.

and *Agarophyton vermiculophyllum* (Ohmi) Gurgel, J. N. Norris et Fredericq (15-33%) (Villanueva *et al.*, 2010b, as *G. vermiculophylla*). The agar yield showed seasonal differences, with lower values during the rainy season, and lower salinity (Figs. 2 and 6). The obtained results agree with previous studies on other Gracilarieae tribe species in Tropical or Temperate-Tropical regions, where a low agar yield was obtained in the rainy season when both, a lower salinity and water temperature occur. This has been explained as an effect by rainwater leach to the coast that impacts the physiological performance of the seaweed (e.g., ionic balance, cellular exudation) (John & Asare, 1975; Buriyo & Kivaisi, 2003; Orduña-Rojas *et al.*, 2008). In contrast, high agar yields have been reported for the rainy season and as the result of the agar deposition between the cell walls to increase structural support in low-salinity waters (Bird, 1988; Sornalakshmi, 2017).

Regarding the gel strength of *G. parvispora* native agar, this was higher (e.g., Ballenato 367.3 ± 14.2 g cm^{-2}) than other reports from Tropical Gracilarieae tribe species, such as *G. cervicornis* (Turner) J. Agardh (< 50 g cm^{-2}) (Freile-Pelegrín & Murano, 2005), *G. parvispora* (170 g cm^{-2}) (Vergara-Rodarte *et al.*, 2010, as *G. vermiculophylla*), and *Hydropuntia edulis* (S. G. Gmelin) Gurgel & Fredericq (78.05-166.66 g cm^{-2}) (Lee *et al.*, 2016; as *G. edulis*). The negative correlation that was detected between gel strength and agar yield (r = –0.52, Table 3) has been described for other Gracilarieae tribe species such as *Crassiphycus crassissimus* (P. Crouan & H. Crouan) Gurgel, J. N. Norris & Fredericq (Freile-Pelegrín & Murano, 2005; as *G. crassissima*) in a Tropical region, and *G. parvispora* in a Temperate-Tropical region (Arvizu-Higuera *et al.*, 2008; Krueger-Hadfield *et al.* 2016; as *G. vermiculophylla*). Interspecific differences also have been reported for gel strength and attributed to structural differences among agar molecules that define the gelling properties of agar (substitution patterns, molecular weight, and distribution) (Oyieke, 1993; Marinho-Soriano *et al.*, 2001; Lee *et al.*, 2016)

Regarding the gelling temperature of native agar from *G. parvispora*, the obtained values were in accordance with standard values (32°C to 39°C), while hysteresis and melting temperature were lower than the standard values (hysteresis > 45°C, melting temperature ≥ 85°C) (Murano, 1995; Armisén & Galatas, 2000). However, the hysteresis obtained for Ballenato gels was close to the required standards. In general, the gelling temperature is influenced by the presence of methyl groups on the agar molecular structure, the melting temperature depends on the molecular weight (higher molecular weight results in higher melting temperature), and high hysteresis derives from high agarose content (Murano, 1995; Armisén & Galatas, 2000).

The mean 3,6-AG content obtained for *G. parvispora* agar was 33.5 ± 4.5 %, which ranged from 28.6 ± 4.4 % to 39.2 ± 4.5 %. This range agree with those obtained for other Gracilarieae tribe species (from Temperate to Tropical regions) (25.4-40.3%), including *G. tenuistipitata* var. *liui* Zhang & M. Xia (25.3%) from Temperate-Tropical regions, and *Hydropuntia edulis* (as *G. edulis*) (37.5%) and *G. gracilis* (Stackhouse) Steentoft, L. M. Irvine & Farnham (40.3%) from Tropical regions (Rebello *et al.*, 1997). The sulfate content obtained for *G. parvispora* in the dry season (4.75 ± 0.15%) falls in the range of values cited for the genus

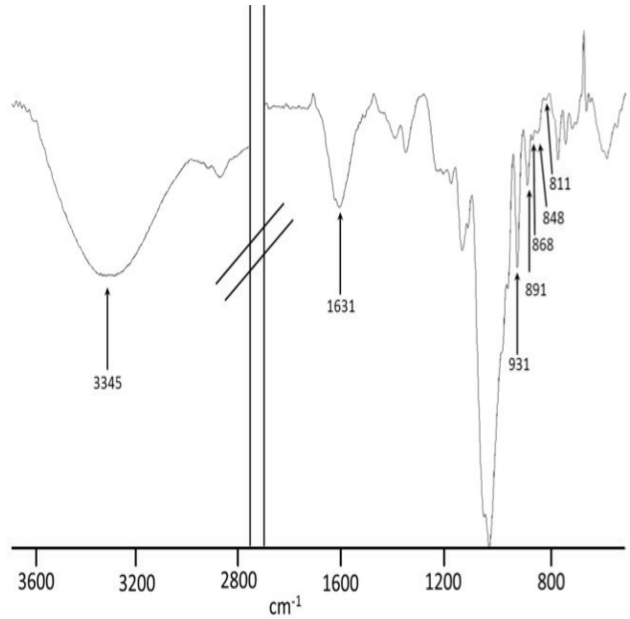

Figure 8. FTIR-ATR spectrum of a native agar sample obtained from Gracilaria parvispora representative of the dry season (Paredón locality).

Gracilaria (< 10%) (Murano, 1995), while the sulfate content obtained in the rainy season is higher than those values (12.69 ± 0.21%). The negative correlation found between 3,6-AG and sulfate content (r = –0.52, Table 3) has also been described previously for Gracilarieae tribe species (Freile-Pelegrín & Robledo, 1997b). Finally, the seasonal variation of 3,6-AG and sulfate content observed agrees with the values reported for several *Gracilaria* species (Murano, 1995; Hung *et al.*, 2000; Buriyo & Kivaisi, 2003).

For the FTIR-ATR spectroscopy analysis, the spectra show absorption bands at 3330 cm^{-1} assigned to OH groups, and a weak signal at 2943 cm^{-1} assigned to CH_2 groups. The band at 2896 cm^{-1} is attributed to CH_3 groups; the highest intensity of this band with respect to that for CH_2 suggests the presence of methyl esters (O-CH_3). The signals observed in the range of 1380-1200 cm^{-1} are related to the vibrational modes of sulfate groups, mainly the bands around 1245 cm^{-1}. This band is related to the presence of α-L-galactose-6-sulfate, a precursor of 3,6-AG which gives a strong signal at 930 cm^{-1} corresponding to the vibrational modes of the C-O-C bridge present in this monomer. Weak signals observed at 868 cm^{-1} correspond to L-galactose-6-sulfate; the bands at 850 and 820 cm^{-1} are attributed to vibrations of the sulfate group of D-galactose-4-sulfate (Figs. 8 and 9) (Praiboon *et al.*, 2006; Villanueva *et al.*, 2010a; Barros *et al.*, 2013; Guerrero *et al.*, 2014).

In sulfated polysaccharides such as agar, the position of the sulfate groups can be determined by the absorption bands in the range of 800-880 cm^{-1}. In the literature, the bands at 845 cm^{-1} and a small shoulder at 830 cm^{-1} are attributed to vibrations of the 4-O-sulfate and 2-O-sulfate bonds of D-galactose; the signals at 820 and 805 cm^{-1} are attributed to substitutions in the C6 and C2 carbons of L-galactose and 3,6-AG, respectively. The presence of sulfates in carbon 4 of L-galactose is related to gel strength, a parameter of agar quality (Tako, 2015). This signal decreases with alkaline treatment (Fig. 9), and the gel strength increases; other bands that are affected and that are representative of the presence of sulfates are those in the range of 1380-1200 cm^{-1}, specifically those around 1245 cm^{-1}. When L-galactose-6 sulfate loses sulfate due to alkaline treatment, this band decreases in intensity; L-galactose-6 sulfate gives way to 3,6-AG, and the band at 930 cm^{-1} increases and, with this, the gel strength (Mazumder *et al.*, 2002; Viana *et al.*, 2004; Heydari *et al.*, 2014).

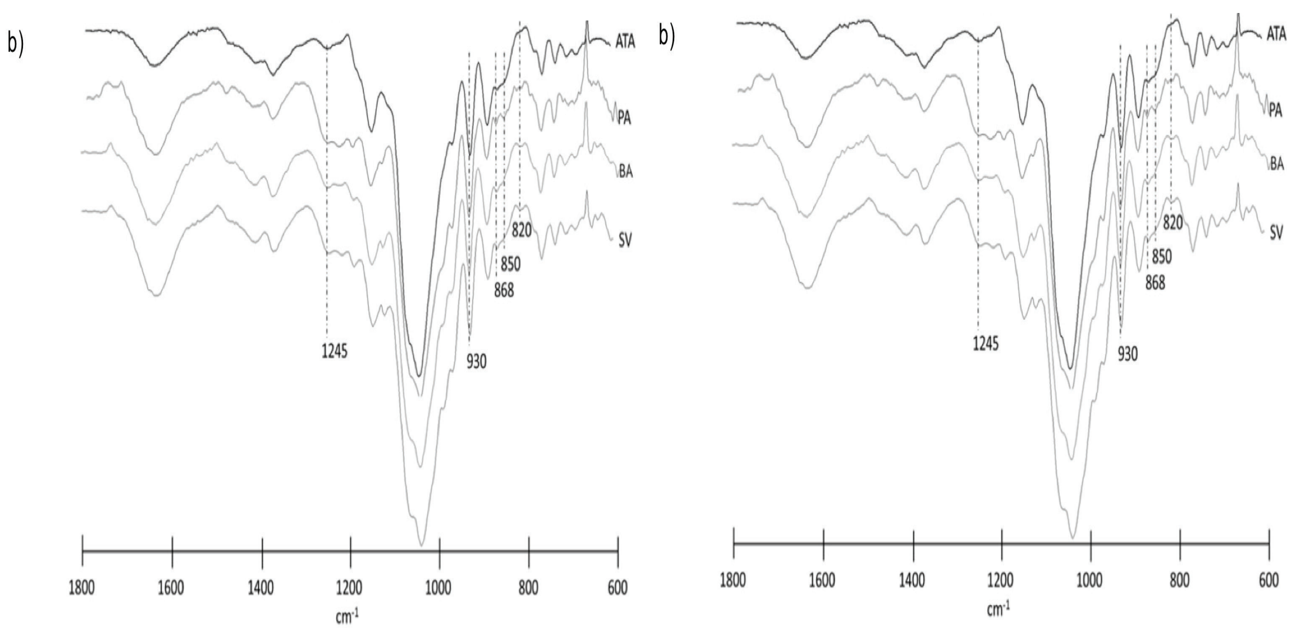

Figure 9. Comparative FTIR-ATR spectra of the native agar obtained from Gracilaria parvispora collected in the localities of Paredón (PA), San Vicente (SV) and Ballenato (BA), in the rainy season (a) and dry season (b).

The native agar of *G. parvispora* shows a pattern of substitution of sulfate groups similar to those obtained from other sources previously reported (Praiboon *et al.,* 2006). Moreover, it does not show considerable differences between either samples collected in a different area or at different times.

In conclusion, the properties of native agar from *G. parvispora* are similar to those for other Gracilarieae tribe species distributed in Tropical and Temperate-Tropical regions. The agar yield was relatively low, but it is in the range previously cited for *G. parvispora* and for other *Gracilaria* species. The gel strength of *G. parvispora* native agar from TMP is higher than reported for other Gracilarieae tribe species; however, the gel strength does not comply with the standards required for commercial use. Melting temperature and hysteresis values were low and close to the characteristic values of agar gels. The gelling temperature was lower than those established for the genus. The mean values for 3,6-AG and sulfate content are in the range reported for other Gracilarieae tribe species. In particular, the best native agar properties of *G. parvispora* were found for gels obtained from Ballenato samples and during the dry season. The gel strength and melting temperature were close to standard values. Moreover, in order to improve gel properties, it may be necessary to carry out an alkaline treatment as proposed by Freile-Pelegrín and Robledo (1997a) and Arvizu-Higuera *et al.* (2008). Finally, a longer sampling period is suggested to follow the continuous agar variations along the time.

ACKNOWLEDGMENTS

EFRA express their thanks for the fellowship granted under the PRODEP-SEP project titled “Especies exóticas de México”. MJTM is grateful for the scholarships sponsored by the previously cited project. GHC, DLAH, MMO and YERM thank the COFAA and EDI programs of the IPN for the fellowship awarded.

REFERENCES

Abbott, I. A. 1985. Sección IV. *Gracilaria*. *In:* Abbott, I. A. & J. N. Norris (eds.). *Taxonomy of Economic Seaweeds with Reference to Some Pacific and Caribbean Species*. California Sea Grant College Program, La Jolla, CA, pp. 115-156.

Armisén, R. 1995. World-wide use and importance of *Gracilaria*. *Journal of Applied Phycology* 7: 231-243.

Armisén, R. & F. Galatas. 1987. Production, properties and uses of agar. *In:* McHugh, D. J. (ed.). *Production and Utilization of Products from Commercial Seaweeds*. FAO Fisheries Technical Paper 288. FAO, Rome, pp. 1-57.

Armisén, R. & F. Galatas. 2000. Agar. *In:* Phillips, G. & P. Williams (eds.). *Handbook of Hydrocolloids*. CRC, Boca Raton, FL, pp. 21-40.

Arvizu-Higuera, D. L., Y. E. Rodríguez-Montesinos, J. I. Murillo-Álvarez, M. Muñoz-Ochoa & G. Hernández-Carmona. 2008. Effect of alkali treatment time and extraction time on agar from *Gracilaria vermiculophylla*. *Journal of Applied Phycology* 20: 515-519.

Barros, F. C. N., D. C. da Silva, V. G. Sombra, J. S. Maciel, J. P. Feitosa, A. L Freitas & R. C. de Paula. 2013. Structural characterization of polysaccharide obtained from red seaweed *Gracilaria caudata* (J. Agardh). *Carbohydrate Polymers* 92(1): 598-603.

Bird, K. T. 1988. Agar production and quality from *Gracilaria* sp. strain G-16: effects of environment factors. *Botanica Marina* 31: 33-39.

Buriyo, A. S. & A. K. Kivaisi. 2003. Standing stock, agar yield and properties of Gracilaria salicornia harvested along the Tanzanian coast. Western Indian Ocean Journal of Marine Science 2: 171-178.

Callaway, E. 2015. Lab staple agar runs low, dwindling seaweed harvest imperils reagent essential for culturing microbes. *Nature* 528: 171-172.

Camacho, O. & G. Hernández-Carmona. 2012. Fenología y alginatos de dos especies de *Sargassum* de la costa Caribe de Colombia. *Ciencias Marinas* 38(2): 381-393.

Contreras, F., O. Castañeda & R. Torres. 1997. Hidrología, nutrientes y productividad primaria en lagunas costeras del estado de Oaxaca, México. *Hidrobiológica* 7: 9-12.

Dobson, A. J. 2002. *An introduction to generalized linear models*. Chapman & Hall/CRC, New York. 255 p.

Dreckmann, K. M. 1999. El género *Gracilaria* (Rhodophyta) en el Pacífico Centro-Sur de México. I. *Gracilaria parvispora* I. A. Abbott. *Hidrobiológica* 9(1): 71-76.

Freile-Pelegrín, Y. & E. Murano. 2005. Agars from three species of *Gracilaria* (Rhodophyta) from Yucatán Peninsula. *Bioresource Technology* 96: 295-302.

Freile-Pelegrín, Y. & D. Robledo. 1997a. Effects of season on the agar content and chemical characteristics of *Gracilaria cornea* from Yucatan, Mexico. *Botanica Marina* 40: 285-290.

Freile-Pelegrín, Y. & D. Robledo. 1997b. Influence of alkali treatment on agar from *Gracilaria cornea* from Yucatan, Mexico. *Journal of Applied Phycology* 9: 533-539.

García-Rodríguez, L. D., R. Ríosmena-Rodríguez, S. Y. Kim, M. López-Meyer, J. Orduña-Rojas, J. M. López-Vivas & M. Boo. 2013. Recent introduction of *Gracilaria parvispora* (Gracilariales, Rhodophyta) in Baja California, Mexico. *Botanica Marina* 56(2): 143-150.

Gómez-Ordóñez, E. & P. Rupérez. 2011. FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds. *Food Hydrocolloids* 25: 1514-1520.

Guerrero, P., A. Etxabide, I. Leceta, M. Peñalba & K. de la Caba. 2014. Extraction of agar from *Gelidium sesquipedale* (Rhodopyta) and surface characterization of agar based films. *Carbohydrate Polymers* 99: 491-498.

Gurgel, C. F. D., J. N. Norris, W. E. Schmidt, H. N. Le & S. Fredericq. 2018. Systematics of the Gracilariales (Rhodophyta) including new subfamilies, tribes, subgenera, and two new genera, *Agarophyton gen. nov.* y *Crassa gen. nov. Phytotaxa* 374(1): 1-23.

Hair, J. F., R. E. Anderson, R. L. Tatham & W. C. Black. 1999. *Análisis Multivariante*. Prentice Hall, Madrid. 799 p.

Heydari, M., M. A. Nematollahi, A. Motamedzadegan, S. Hashem & S. V. Hosseini. 2014. Optimization of the yield and quality of agar from *Gracilariopsis persica*. *Bulletin of Environmental, Pharmacology and Life Sciences* 3(3): 33-40.

Hung, L. D., H. Q. Nang & N. Q. Buu. 2000. Chemical composition of sulfated galactans agar from some *Gracilaria* species growing along the coast of southern Vietnam. *Journal of Chemistry* 38: 80-83.

John, D. M. & S. O. Asare. 1975. A preliminary study of the variations in yield and properties of phycocolloids from Ghanaian seaweeds. *Marine Biology* 30: 325-330.

Krueger-Hadfield, S. A., G. Hernández-Carmona, R. Terada, J. M. López-Vivas & R. Riosmena-Rodríguez. 2016. New record of the non-native seaweed *Gracilaria parvispora* in Baja California - A note on Vergara-Rodarte *et al.* (2016). *Cryptogamie, Algologie* 37(4): 257-263. DOI:10.7872/crya/v37.iss4.2016.257

Lahaye, M. & W. Yaphe. 1988. Effects of seasons on the chemical structure and gel strength of *Gracilaria pseudo-verrucosa* agar (Gracilariaceae, Rhodophyta). *Carbohydrate Polymers* 8: 285-301.

Lee, W. K., P. E. Lim, S. M Phang, P. Namasivayam & C. L. Ho. 2016. Agar properties of *Gracilaria* species (Gracilariaceae, Rhodophyta) collected from different natural habitats in Malaysia. *Regional Studies in Marine Science* 7: 123-128.

León-Tejera, H. & J. González-González. 2000. Macroalgal communities from Laguna Superior, Oaxaca. *In:* Munawar, M., S. G. Lawrence, I. F. Munawar & D. F. Malley (eds.). *Aquatic Ecosystems of Mexico. Status and Scope*. Backhuys Publishers, Leiden, pp. 323-334.

Marinho-Soriano, E., T. S. Silva & W. S. C. Moreira. 2001. Seasonal variation in the biomass and agar yield from *Gracilaria cervicornis* and *Hydropuntia cornea* from Brazil. *Bioresource Technology* 77: 115-120.

Mateo-Cid, L. E. & A. C. Mendoza-González. 2012. Algas marinas bentónicas de la costa noroccidental de Guerrero, México. *Revista Mexicana de Biodiversidad* 83: 905-928.

Matsuhiro, B. 1996. Vibrational spectroscopy of seaweed galactans. *Hydrobiologia* 326(1): 481-489.

Mazumder, S., P. K. Ghosal, C. A. Pujol, J. Carlucci, B. Damonte & B. Ray. 2002. Isolation, chemical investigation and antiviral activity of polysaccharides from *Gracilaria corticata* (Gracilariaceae, Rhodophyta). *International Journal of Biological Macromolecules* 31: 87-95.

McHugh, D. J. 2002. *Prospects for seaweed production in developing countries*. FAO Fisheries Circulars. No. 968. FAO, Rome. 28 p.

McHugh, D. J. 2003. *A guide to the seaweed industry*. FAO Fisheries Technical Paper No. 441. FAO, Rome. 105 p.

Mendoza-González, C. A. & L. E. Mateo-Cid. 1999. Adiciones a la ficoflora marina bentónica de las costas de Oaxaca, México. *Polibotánica* 10: 39-58.

Menges, F. 2020. Spectragryph, optical spectroscopy software. Available online at: https://www.effemm2.de/spectragryph/down.html (downloaded December 15, 2020).

Murano, E. 1995. Chemical structure and quality of agars from *Gracilaria*. *Journal of Applied Phycology* 7: 245-254.

Orduña-Rojas, J., K. Y. García-Camacho, P. Orozco-Meyer, R. Ríosmena-Rodríguez, I. Pacheco-Ruíz, J. A. Zertuche & A. E. Melling-López. 2008. Agar properties of two species of Gracilariaceae from the Gulf of California. *Journal of Applied Phycology* 20: 169-175.

Oyieke, H. A. 1993. The yield, physical and chemical properties of agar from *Gracilaria* species (Gracilariales, Rhodophyta) of the Kenya Coast. *Hydrobiologia* 260/261: 613-620.

Pereira, L., S. F. Gheda & P. J. A. Ribeiro-Claro. 2013. Analysis by vibrational spectroscopy of seaweed polysaccharides with potential use in food, pharmaceutical, and cosmetic industries. *International Journal of Carbohydrate Chemistry* 2013: 537202. DOI:10.1155/2013/537202

Praiboon, J., A. Chirapart, Y. Akakabe, O. Bhumibhamon & T. Kajiwara. 2006. Physical and chemical characterization of agar polysaccharides extracted from the Thai and Japanese species of *Gracilaria*. *Science Asia* 32(1): 11-17.

Rebello, J., M. Ohno, H. Ukeda, H. Kusunose & M. Sawamura. 1997. 3,6 anhydrogalactose, sulfate and methoxyl contents of commercial agarophytes from different geographical origins. *Journal of Applied Phycology* 9: 367-370.

Sornalakshmi, V. 2017. Effects of season on the yield and properties of agar from *Gracilaria corticata. International Journal of Sciences, Engineering and Management* 2(12): 206-211.

Tako, M. 2015. The principle of polysaccharide gels. *Advances in Bioscience and Biotechnology* 6(1): 22-36.

Tapia-García, M., M. C. García-Abad, F. E. Penagos-García, J. L. Moreno-Ruíz, L. G. Juárez-Hernández, J. M. Ramírez-Gutiérrez & D. Herrera-Olayo. 2011. Subsistemas hidrológicos de la laguna Mar Muerto Oaxaca-Chiapas, México. *Lacandonia* 5(1): 97-112.

Terho, T. T. & K. Hartiala. 1971. Method for determination of sulfate content of glycosaminoglycans. *Analytical Biochemistry* 41: 471-476.

Vergara-Rodarte, M. A., G. Hernández-Carmona, Y. E. Rodríguez-Montesinos, D. L. Arvizu-Higuera, R. Riosmena-Rodríguez & J. I. Murillo-Alvarez. 2010. Seasonal variation of agar from *Gracilaria vermiculophylla*, effect of alkali treatment time, and stability of its Colagar. *Journal of Applied Phycology* 22: 753-759.

Viana, A. G., M. D. Noseda, M. E. R. Duarte & A. S. Cerezo. 2004. Alkali modification of carrageenans. Part V. The iota-nu hybrid carrageenan from *Eucheuma denticulatum* and its cyclization to iota-carrageenan. *Carbohydrate Polymers* 58(4): 455-460.

Villanueva, R. D., J. B. Romero, A. L. R. Ragasa & M. N. E. Montaño. 2010a. Agar from the red seaweed, *Laurencia flexilis* (Ceramiales, Rhodophyta) from northern Philippines. *Phycological Research* 58(2): 151-156.

Villanueva, R. D., A. M. M. Sousa, M. P. Gonçalves, M. Nilsson & L. Hilliou. 2010b. Production and properties of agar from the invasive marine alga, *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta). *Journal of Applied Phycology* 22: 211-220.

Yaphe, W. & G. P. Arsenault. 1965. Improved resorcinol reagent for the determination of fructose and of 3,6-anhydrogalactose in polysaccharides. *Analytical Biochemistry* 13: 143-148.

Zar, J. H. 2010. *Biostatistical Analysis*. Pearson, New Jersey. 994 p.