Research Article

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Seasonal patterns of growth, alginate content and block structure of the alien invader *Sargassum muticum* (Fucales, Ochrophyta) from the Atlantic coast of Morocco

https://doi.org/10.1515/bot-2021-0050
Received May 29, 2021; accepted October 20, 2021;
published online December 7, 2021

Abstract: In the present study, alginate yield and composition were investigated during the seasonal life cycle of the alien brown alga *Sargassum muticum* harvested from the Atlantic coast of Morocco. Alginate yield ranged from 11.14% in winter to 25.62% in spring/early summer, coinciding with maximum vegetative growth. Monthly monitoring of the alginate block structure showed that the highest mannuronate (M)/guluronate (G) ratio was recorded during the maximum development of *S. muticum*, before sexual maturity and during resumption of vegetative growth, giving maximum flexibility to the alga. The unusually high molar monad fractions (F_G) and dyad fractions (F_{GG}) of guluronic acid in late summer/early autumn appeared to be related to stiffness of senescent thalli. Rheological characterisation showed that the alginate of *S. muticum* exhibited pseudoplastic behaviour, with the highest apparent viscosities measured in late summer/early autumn when the G blocks dominated the alginate structure. This study suggests that *S. muticum* could be exploited as an alginophyte for commercial applications. The best harvest time is May-June, which corresponds to the highest alginate yield, maximum thallus growth, and largely completed sexual reproduction, ensuring sustainable exploitation of the species.

Keywords: alginate; chemical characterisation; rheology; *Sargassum muticum*; seasonal variation.

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1 Introduction

Seaweeds are harvested by coastal communities worldwide for food and other purposes (Padam and Chye 2020). In the Western hemisphere, seaweeds are exploited primarily for their content of high molecular weight polysaccharides (i.e. phycocolloids) (Pangestuti and Kim 2015). These hydrocolloids are used for various commercial purposes in pharmaceuticals, food technology, biotechnology, cosmetics and engineering (Venugopal 2008). The annual global production of phycocolloids is approximately 100,000 t, with a gross market value of US$ 1 billion per year (Pangestuti and Kim 2015). This huge production results from the growing global demand for phycocolloids. The main producing countries for phycocolloids are in the Asia-Pacific region, with an estimated production of 31,844 t for alginate, 27,200 t for carrageenan, and 10,000 t for agar in 2015, accounting for more than half of global hydrocolloid production (Gomez et al. 2020). Alginate is the main polysaccharide structuring the cell walls of brown algae; it is composed of mannuronic (M) and guluronic (G) acids with a variable M/G ratio depending on species, age, and thallus

part (Stiger-Pouvreau et al. 2016). The M/G ratio and the distribution of G and M blocks in the chain greatly affect the physiological and rheological properties of alginate (Sugiono et al. 2019).

Alginate is widely used as a gelling agent, thickener, stabiliser or emulsifier in the food, pharmaceutical and textile industries (Hernández-Carmona et al. 2013; Puscaselu et al. 2020). The biocompatibility, biodegradability, and non-toxicity of alginate make it a biomaterial with various applications in the pharmaceutical and biomedical industries, e.g. drug delivery, wound healing and tissue engineering (Puscaselu et al. 2020; Ziółkowska et al. 2020). Alginate is also of interest in biomedicine in the form of hydrogels, microspheres, microcapsules, sponges, foams, fibres, and porous scaffolds (Łabowska et al. 2020). In addition, the alginate gel matrix has been used to encapsulate probiotics, functional proteins, fish oil and micronutrients (Bokkhim et al. 2016; Krasaekoopt et al. 2006; Sohail et al. 2011). Alginate is mainly obtained from *Laminaria hyperborea*, *Laminaria digitata*, *Saccharina japonica*, *Macrocystis pyrifera*, *Ascophyllum nodosum*, *Ecklonia maxima*, *Lessonia nigrescens*, *Durvillaea antarctica* and *Sargassum* spp. (Draget and Taylor 2011). In the last decade, the harvest of *Macrocystis pyrifera* has declined sharply, with a decrease in harvested biomass from 35,000 t (1999) to 5000 t (2009) after the closure of the International Specialty Products plant in San Diego (Hernández-Carmona et al. 2013). Similar changes occurred for *Ascophyllum*, whose production decreased from 13,500 t in 1999 to 2000 t in 2009 (Bixler and Porse 2011). Conversely, exploitation of the genus *Sargassum* as a raw material for the alginate industry has increased for example on the Indian coasts (Ganesan et al. 2019).

The alien *Sargassum muticum* forms well-established populations on the Moroccan Atlantic coast, where thalli can grow up to 5 m long and form dense mats that dominate the native macroalgae in over 90% of tidal pools. In this context, the widespread canopies of the invasive *Sargassum muticum* could be a promising biomass source for the alginate industry (Belattmania et al. 2017; El Atouani et al. 2016). However, there are no data on the seasonal variability of the content and physicochemical properties of alginates of this species. Accordingly, this study sought to address this lack of data by pursuing the following objectives: i) determination of alginate content during the seasonal life cycle of *S. muticum*, ii) investigation of alginate quality through spectroscopic characterisation (FTIR and ^{1}H NMR) and rheological behaviour of monthly extracted alginates, iii) determination of the most suitable harvesting season for sustainable exploitation of *S. muticum* on the Atlantic coast of Morocco.

2 Materials and methods

2.1 Sampling of the biomass

Sargassum muticum (Yendo) Fensholt samples were collected from the Northwestern Atlantic coast of Morocco (33° 14′47.53″N 8° 32′31.9″W). Alginate yield and composition were monitored monthly from January to December 2017, as was thalli phenology (i.e., length, dry weight and fertility index) during the annual life cycle. Thalli were rinsed several times in the laboratory with fresh and distilled water to remove salt, epiphytes and adhering debris. The biomass was air-dried for 4–5 days and then dried in an oven at 60 °C to constant weight before being used for alginate extraction.

2.2 Alginate extraction

Alginate was extracted according to the modified procedure of Calumpong et al. (1999). Dried biomass of *S. muticum* (25 g) was hydrated in 2% formalin for 24 h, washed with distilled water and soaked in 0.2 N HCl for 24 h. Algal samples were then washed with distilled water and extracted with 2% Na_2CO_3 for 24 h, filtered through three layers of cheesecloth and the filtrate collected by centrifugation (4500*g*, 20 min). The extract was discarded and the procedure was repeated for the solid residue. The entire filtrate was precipitated with three volumes of 95% ethanol. The resulting sodium alginate fibres were washed with acetone and dried in an oven at 50 °C until constant weight. The alginate yield was expressed as a percentage of the initial dry weight of the alga (% dw).

2.3 Spectroscopic characterisation

2.3.1 FT-IR spectroscopy: FTIR spectral measurements of the dried sodium alginate samples (50 °C for 3 h) were performed using a Thermo Scientific Nicolet Impact 400D FT-IR Spectrometer (Nicolet Instrument Co., Madison, USA). Spectra were scanned between 4000 and 600 cm^{-1} in attenuated total reflectance (ATR) mode. For each sample, a total of 32 scans were averaged with a resolution of 4 cm^{-1}. Subsequently, the IR spectra were processed with OMNIC software (Nicolet, Madison, USA).

2.3.2 ^{1}H NMR spectroscopy: ^{1}H NMR spectra of sodium alginate solutions in D_2O were recorded with a AV II 400 MHz, 9.4T spectrometer (Proton Larmor frequency of 400.33 MHz, Bruker Corporation, Billerica, MA, USA) using a 5 mm Triple Resonance Broadband Inverse (TBI) probe (Bruker Corporation, Billerica, MA, USA) at 343 K. The spectra consisted of 16 K size of free induction decay covering a sweep width of 4800 Hz. A presaturation was applied during the relaxation delay and mixing time. The exponential multiplication apodization functions were applied in one dimension with 0.5 for line broadening prior to Fourier transformation. The number of scans was 32 transients.

2.4 Rheological characterisation

The rheological behaviour of the sodium alginate solutions was assessed by a Haake Mars II rheometer (Thermo Fisher Scientific Inc.,

Waltham, MA, USA) with parallel plate measuring geometry (35 mm diameter, 1 mm gap) and data processing using Haake RheoWin DataManager software version 3.12 (Thermo Fisher Scientific Inc). The viscous properties of sodium alginate solutions (2% w/v) were determined by steady-shear flow tests in the shear rates range of 12–600 s^{-1}.

2.5 Statistical analyses

The experimental error was determined for five replicate assays and expressed as standard deviation (SD). A one-way ANOVA was performed to test the effect of sampling time on alginate yield, using the month of sampling (12 levels) as a fixed factor. Prior to ANOVA analysis, homogeneity of variances and normality were checked using Kolmogorov–Smirnov's test and Levene's test respectively and data were log $(x + 1)$ transformed to remove heteroscedasticity. Spearman's correlation coefficient was used to examine the relationships between alginate content, length, dry weight and fertility of thalli.

3 Results and discussion

3.1 Alginate content

The alginate yield of *S. muticum* showed significant temporal fluctuations. It gradually increased from January onwards (20.44% dw) and reached the highest value in May (25.62%). Thereafter, a marked decrease was observed in summer, with a minimum of 11.14% in September (Figure 1a). The temporal variations in alginate yield followed the same trend as the algal life cycle (Figure 1a,b). A significant positive correlation was found between alginate yield and thallus length (Table 1), and alginate yield was also positively correlated with thallus dry weight ($r = 0.515$, $p < 0.01$). Thallus length and dry weight had peak values of 122 ± 2.78 cm and 214 ± 14.47 g, respectively, in summer (June-July) and decreased sharply in September (Figure 1b). Fertility index was also correlated with alginate content ($r = 0.295$, $p < 0.05$). Indeed, the Fertility index gradually increased from March and reached its maximum (1) in early summer (Figure 1b).

Table 1: Correlation coefficients (Spearman's rho) between alginate yield and thallus size, dry weight and fertility of *Sargassum muticum*.

	Thallus size	Thallus dry weight	Fertility index
Alginate yield	0.455**	0.515**	0.295*

**$p < 0.01$; *$p < 0.05$.

The content and quality of alginate are influenced by many factors such as species, harvest period, tissue type, and age (Haug et al. 1974; Łabowska et al. 2019; Rinaudo 2014). The peak of alginate recorded in spring is consistent with previous results on other *Sargassum* species. Studies on *S. vulgare* and *S. polyceratium*, from the coasts of Puerto Rico have shown maximum alginate yields (17.5 and 20%,

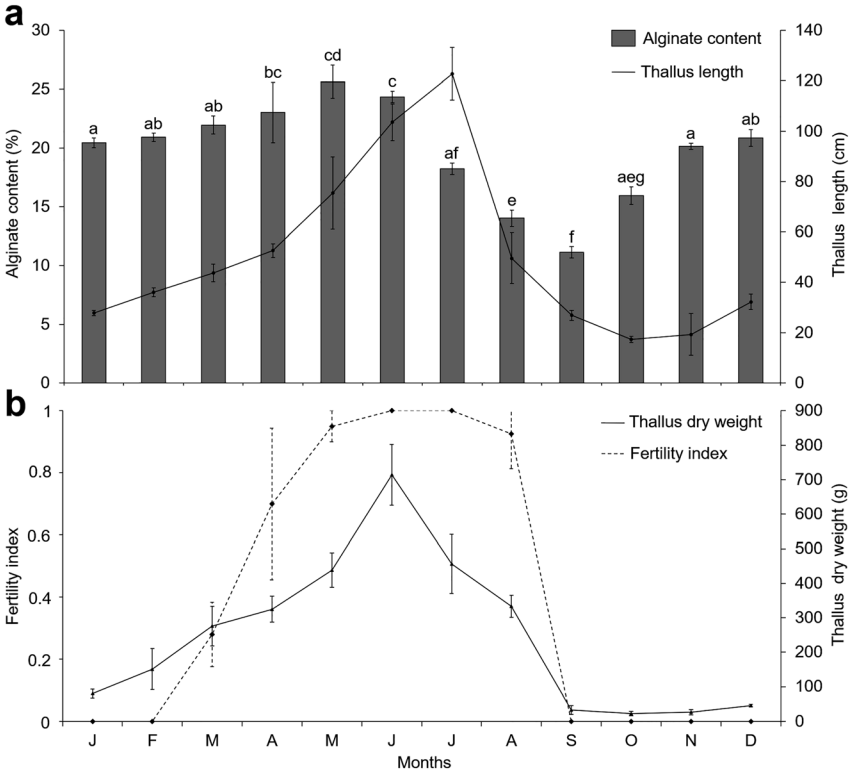

Figure 1: Monthly variation of (a) alginate yield and thallus length and (b) dry weight and fertility index of *Sargassum muticum* from the Moroccan Atlantic coast. Letters indicate significant differences according to one way ANOVA (Tukey test, $p < 0.05$). Error bars: standard deviation ($n = 5$).

respectively) in spring and low yields (7 and 12%) in winter (Aponte de Otaola et al. 1983). Similarly, Kumar and Sahoo (2017) showed that the highest alginate content in *Sargassum wightii* from India was found in March compared to other sampling periods (January, May, July, September and November). In addition, Rodríguez-Montesinos et al. (2008) reported that young thalli of *Sargassum sinicola* from the coasts of Baja California had low alginate content (11.7%) from autumn to winter. As development progressed in spring, the cell wall and intercellular matrix accumulated alginate and reached a maximum of 13.7%. The same phenological sequence and yield behaviour were observed in *S. wightii* (Rao 1969), *S. swartzii*, *S. tenerrimum* (Chauhan 1970) and *S. polycystum* (Chennubhotla et al. 1982).

3.2 Composition of the alginate

3.2.1 FTIR characterisation

The FT-IR spectra of sodium alginates extracted from *S. muticum* during its annual life cycle are shown in Figure 2. The bands at 1666–1680 cm^{-1} were assigned to the asymmetric stretching vibration of O–C–O (Belattmania et al. 2020; Fenoradosoa et al. 2010; Mathlouthi and Koenig 1987; Rashedy et al. 2021). The bands at 1437–1450 cm^{-1} were attributed to the C–OH bending vibration with a contribution from the carboxylate group O–C–O (Ardalan et al. 2018; Fenoradosoa et al. 2010; Papageorgiou et al. 2010). The weak bands at 1066–1076 cm^{-1} have been frequently reported for alginates and can be associated with C–O–C stretching vibrations of the pyranose ring (Papageorgiou et al. 2010; Sakugawa et al. 2004). The intense bands detected at 1001–1012 cm^{-1} could be attributed to OH bending and C–C stretching vibrations of the pyranose ring (Gómez-Ordóñez et al. 2010; Sakugawa et al. 2004). These bands shifted to 982, 991 and 995 cm^{-1} for sodium alginate in July, August and September, respectively, and this shift could be related to the change in the M/G ratio in the summer samples. Similarly, Beratto et al. (2017) pointed out a seasonal variation in the bands associated with the pyranose ring of alginates from *Macrocystis pyrifera* from the Chilean coast. According to Sakugawa et al. (2004), the variation in mannuronic acid concentration resulted in a shift of the peak at 1030 cm^{-1} due to the absorbance of OH bending. The weak bands at 930–935 cm^{-1} could be attributed to the C–O stretching vibration of guluronic acid (Beratto et al. 2017). The bands

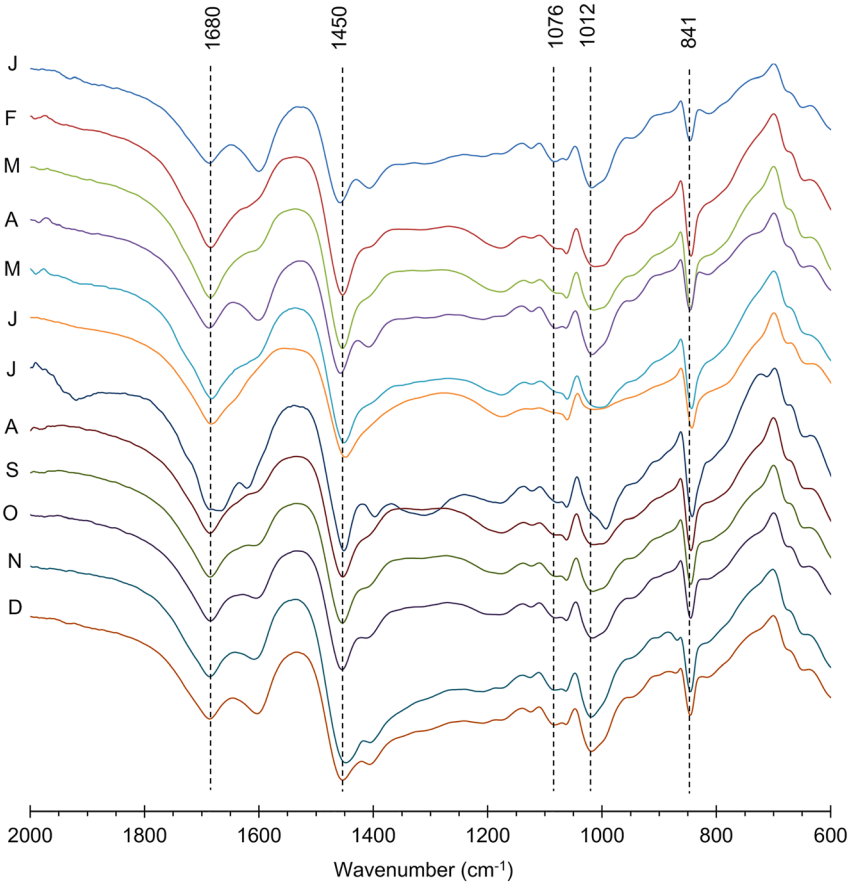

Figure 2: FTIR spectroscopy of sodium alginates extracted from *Sargassum muticum* (January–December).

at 831–841 cm^{-1} are characteristic of mannuronic acid residues (Belattmania et al. 2020; Beratto et al. 2017; Gómez-Ordóñez et al. 2010).

3.2.2 ^{1}H NMR characterisation

The physical properties of alginate depend not only on the M/G ratio but also on the M- and G-block sequences, which show marked structural differences (Haug 1969; Indergaard and Skjak-Braek 1987; Indergaard et al. 1990). The abundance, configuration and proportion of the acid blocks depend on the species (Haug 1969), season (South 1979), age and tissue of the algae (Haug et al. 1974; Indergaard and Skjak-Braek 1987; Indergaard et al. 1990; Larsen et al. 2003). Annual monitoring of the alginate block composition in *S. muticum* using ^{1}H NMR spectra (Figure 3) revealed three key signals corresponding to the anomeric hydrogen of guluronic acid (G) at 5.04–5.06 ppm, the anomeric hydrogens of mannuronic acid (M1) and the H-5 of the alternating blocks (GM-5) overlapping at 4.65–4.74 ppm, and the H-5 of the guluronic acid residues in the homopolymeric G blocks, at 4.45 ppm. The M/G ratio, and the molar fractions of the monads (F_G, F_M) and the dyads (F_{GG}, F_{MM}, F_{MG}, F_{GM}) were calculated from the area of the ^{1}H NMR signals (Figure 3) according to the formula of Grasdalen et al. (1979). The highest M/G ratio (Figure 4) was measured during the full growth phase before thallus maturity and during the resumption of vegetative growth in late autumn, ensuring maximum flexibility of the alga. The high levels of the F_G fraction in late summer-early autumn (55–64%) seem to be responsible for the stiffness of thalli during the senescence phase. This period was also characterised by low content of MM dyads (0–0.9%) and an increase in GG blocks (Figure 4), with the highest proportion (21.1%) in August, when the lateral branches of *S. muticum* detached, leaving only a perennial holdfast from which the branches regenerate in the following winter. In brown algae, the M/G ratio and block structure distribution of alginate are determined by the biosynthesis of the polymer and subsequent genetic and environmental control (Helgerud et al. 2010). Although most biosynthesis studies have been conducted on alginate-producing bacteria, the metabolic pathways mainly involve epimerase activity (Skjåk-Bræk 1992). The early stages of the alginate biosynthetic pathway in brown algae yield polymannuronate (Helgerud et al. 2010; Madgwick et al. 1973). A number of alginate epimerases, both membrane-bound and extracellular, then act on, and progress along, the polymannuronate chain, undergoing M to G epimerisation in specific regions of the polymer in different patterns (Larsen 1981). In fucoids, changes in the M/G ratio of thalli of different ages follow a similar pattern, with older tissues generally richer in G units (Cheshire and Hallam 1985; Indergaard and Skjak-Braek 1987; McKee et al. 1992; Minghou et al. 1984), reflecting the action of C-5 epimerase over time. As with *S. muticum*, observation of the life cycle of *Undaria pinnatifida* showed an increase in alginate production from spring to mid-summer (Skriptsova et al. 2004). The alginate extracted from young blades collected in March contained mainly polymannuronic blocks. The proportion of G-units increased with algal growth in spring and summer (Skriptsova et al. 2004), which is consistent

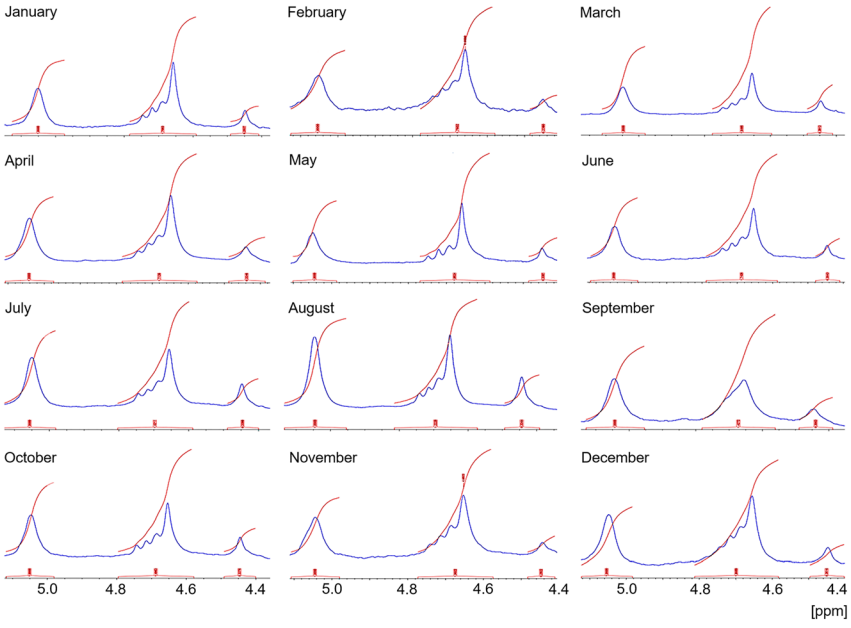

Figure 3: ^{1}H NMR spectra of extracted sodium alginates (January–December) from *Sargassum muticum*.

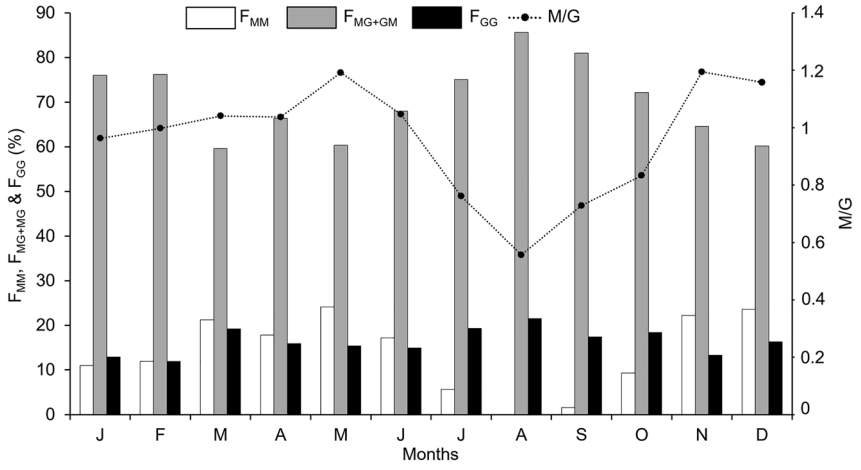

Figure 4: Monthly variation of the mannuronic to guluronic acid ratio (M/G) of the alginate and uronic acid sequence (homoguluronic acid diad fractions; F_{GG}, homomannuronic acid diad fractions F_{MM}, and heteropolymeric fractions F_{MG}, F_{GM}) of *Sargassum muticum*.

with the predicted biosynthetic pattern (Indergaard et al. 1990). In addition, the cell wall of brown algae is colonised by various epiphytic microbial communities (Bengtsson and Øvreås 2010; Corre and Prieur 1990; Staufenberger et al. 2008; Susilowati et al. 2015). Brown algae-associated bacteria can degrade algal polysaccharides such as fucoidans (Nedashkovkaia et al. 2002) and alginate (Brown and Preston 1991). Alginate fragments from wall digestion have been reported to be endogenous elicitors that act as signals and trigger defense responses in brown algae (Küpper et al. 2001, 2002). The oligo-guluronate blocks (G) triggered a much more intense response than the M or mixed MG blocks (Küpper et al. 2001). Therefore, the abundance of guluronic acid F_G and F_{GG} blocks in *S. muticum* at the end of summer may be considered as a response to bacterial proliferation favoured by the decline of the alga at this time. It has been reported that bacteria associated with macroalgae can modulate the growth rate of thalli and the biosynthesis of monosaccharides such as glucuronic acid in the cell wall (Polikovsky et al. 2020).

3.3 Rheological properties

The apparent viscosity of sodium alginate extracted from *S. muticum* was measured over different sampling months (Figure 5). With increasing shear rate, the apparent viscosity of *S. muticum* alginate decreased remarkably, indicating a shear-thinning (pseudoplastic) behaviour. This property resulted from the length and constraint of the hydrated alginate molecules in solution (Onsøyen 1997). When the alginate solution is subjected to a low shear-rate, the alginate molecules are more or less randomly oriented. However, as the shear rate increases, the molecules begin to align themselves and the tangling decreases. Accordingly, the apparent viscosity decreases (Ma et al. 2014; Onsøyen 1997). The flexibility of the polymer chains influences the viscosity of the alginate. The flexibility of the chains is determined by the chemical composition (block structure), as the parts of the chains that contain predominantly G blocks are less flexible than the parts that contain predominantly M blocks, and areas where G blocks monopolize are stiffer than areas where M and G roughly alternate (Smidsrød et al. 1973). The highest values for the apparent viscosity of *S. muticum* alginate (Figure 5) were measured in late summer and early autumn, when the G blocks dominated in the alginate structure. However, the apparent viscosity values decreased from January to May, which was characterised by a high content of MM dyads.

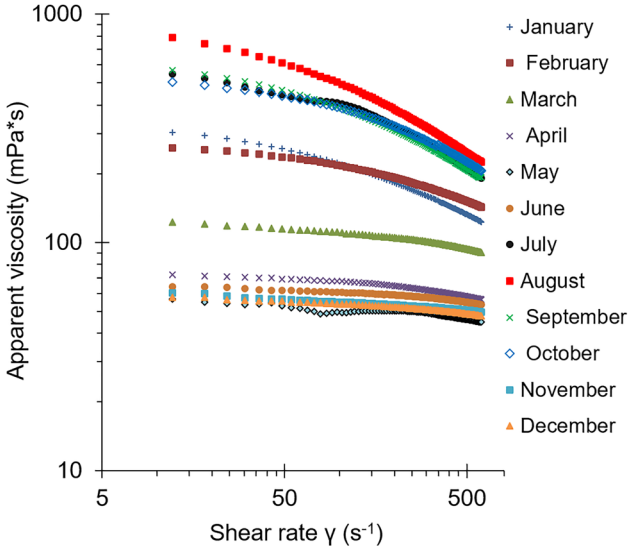

Figure 5: Apparent viscosity of sodium alginate from *Sargassum muticum* in different sampling months.

These results support previous studies in which alginate viscosity of some Fucales has shown a significant positive correlation with the G-block content (Jothisaraswathi et al. 2006; Saraswathi et al. 2003). The seasonal variation in alginate viscosity could simply reflect temporal variations in the local environment (e.g. temperature and nutrients), as the modification of alginic acid biosynthesis is affected by environmental factors (Craigie et al. 1984; Peteiro 2018).

4 Conclusions

The present work has shown that the season of the year is a determining factor for alginate yield and uronic acid composition in *S. muticum*. The maximum alginate yield was obtained in spring/early summer, which coincides with the completion of the vegetative growth phase. There were significant seasonal changes in the composition of the alginate block with the highest M/G ratio in the main growth phase before thallus maturity and during the resumption of vegetative growth, ensuring maximum flexibility of the alga. The unusually high F_G fraction and F_{GG} dyads in late summer/early autumn appeared to be involved in the rigidity of the thalli during the senescence phase. The rheological measurements showed that *S. muticum* alginates exhibit pseudoplastic behaviour with the highest viscosities measured in late summer and early autumn, when the G blocks dominate the alginate structure.

This work demonstrates that *S. muticum* can be a profitable alginophyte species for commercial exploitation. The most suitable period for harvesting on the Moroccan coasts is May-June, which corresponds to the highest alginate yield during the maximum growth of thalli, with sexual reproduction largely completed, ensuring sustainable exploitation of this species. Alternatively, the species should be harvested as part of invasion control during March-April when the thalli are partially mature. In both cases, the proposed commercial activity meets the socio-economic considerations for a seaweed resource that can be effortlessly harvested at low tide, providing employment and income to coastal communities.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: This research was funded by the project VPMA3/DESRS-ANPMA-CNRST "Exploitation de la diversité spécifique et génétique pour une bioraffinerie innovante des algues marines de la côte atlantique marocaine".
Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

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