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Seasonal and spatial compositional variation of the red algae *Mastocarpus stellatus* from the Northern coast of Portugal

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Abstract

This study focusses on the valorization of the red seaweed *Mastocarpus stellatus,* collected in the Northern coast of Portugal, as a natural source of high value compounds due to its beneficial properties. An annual monitoring of the seaweed was performed by determining its lipids, ash, carbohydrates, phycobiliproteins, total phenolic compounds, antioxidant capacity and carrageenan from three different rocky shores located in the north of Portugal. The results showed a seasonal and spatial variability of the studied compounds between October 2018 and September 2019 depending on the climatic variables of temperature, precipitation, and solar radiation. The most productive season coincided with the warmest months, except for carbohydrates and phycobiliproteins, which were promoted in the colder season. The spatial variation also could be explained by the proximity to water channel discharges at the sampling sites. Complementary studies on the carrageenan fraction were conducted in one of the shores due to the high biopolymer content, to determine their carrageenan proportion between the summer and winter period and establish their rheological capabilities for the formulation of gelling matrices. The extracted biopolymers exhibited typical structural and viscoelastic characteristics of *kappa/iota*-hybrid carrageenans, suggesting notably differences depending on the harvest season, which is critically relevant to define future applications.

Keywords Rhodophyta · Biochemical characterization · Seasonality · Bioactivity · Carrageenan · Gelling agent

Introduction

Mastocarpus stellatus or 'false carrageenan moss' is a member of the Rhodophyta, widely distributed on the rocky mid and low-intertidal shore from Norway to the Western Sahara and is an abundant species on the Portuguese coast (Araújo et al. 2009). The use and consumption of this alga as food or ingredient was not very popular in Europe until recently. Nowadays, it is beginning to be exploited as a source of highly valuated compounds, nutrients and bioactives. *Mastocarpus stellatus* exhibits high moisture content (90% fresh weight) and reaches ash values around 25% dry weight, which involves values much higher than those of terrestrial vegetables, making suitable as a food supplement to achieve the recommended daily intakes of some minerals and trace elements (Gómez-Ordóñez et al. 2010). The latter authors also highlighted the notable protein content of this seaweed (21.3%, dry weight), although lower values were identified in other works (15%, dry weight) (Blanco-Pascual et al. 2014a). The red color of this seaweed is attributed to pigments such as phycobiliproteins, considered a light-harvesting protein complex founds in chloroplast of red seaweeds (Nguyen et al. 2017) and their extracts are used as natural dye, food colorant, dietary supplements and are incorporated to functional products (Mysliwa-Kurdziel & Solymosi, 2016; Fleurence et al., 2018). *Mastocarpus stellatus* is considered a reliable source of hybrid carrageenans. This is the main water-soluble biopolymer accounting for around 10%

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(dry weight) of this seaweed (Pereira and Van De Velde, 2011, and can be structured in three forms (*kappa, iota* and *lambda*) which exhibit different gelling properties from liquid to viscous condition (Moreira et al. 2016). Carrageenans are characterized as a powerful gelling agent that have wide applications in industry (Sanz et al. 2021; Guo et al. 2022), due to their remarkable biodegradability and durability properties, and easy extraction. Nowadays, they are used in the food industry, pharmacology and cosmetology, included as an alternative to plastic films (Blanco-Pascual et al. 2014b; Sudhakar et al. 2018; Gamero-Vega et al. 2020). Another feature of this seaweed is its low lipid content (1–3%), making it ideal for human consumption (Sudhakar et al. 2018) and low-fat diets. Apart from being a good source of antioxidants, components that prevent damage caused by oxidation processes as well as mitigate physiological activities, having anti-carcinogenic, antiviral, antibacterial and anti-inflammatory properties (Corsetto et al. 2020).

Monitoring of *M. stellatus* is convenient to determine the dynamics of distribution and abundance across space and seasons to support a sustainable extraction of this resource (Ramírez et al. 2003) and to be profitable for commercial proposes, due to its high content of bioactives and valuable components potentially beneficial for human health, which has markedly increased the extraction of the algae in recent years (Melo et al. 2021). The composition, biomass and productivity of seaweeds seems to exhibit a significant spatial–temporal variation, mainly influenced by external conditions and stress factors (Gaspar et al. 2017). Previous studies evaluated the geographic and temporal variation on the chemical characteristics of *M. stellatus* from the Atlantic coast of Portugal and Spain (Pereira and Van De Velde 2011; Hilliou et al. 2012; Tasende et al. 2013; Pereira et al. 2014), as well as other authors provided information regarding this patterns from different algal species with economic importance under different environmental conditions (Pinho et al. 2016; Véliz et al. 2019; Borges et al. 2020; Afonso et al. 2021), being fundamental for the management and limits in their harvesting.

In order to improve this knowledge, this work is aimed to 1) evaluate the temporal and spatial behavior of *M. stellatus* from three sites on Northern Portugal, in terms of nutritional composition (ash, lipids, carbohydrates, and carrageenan), phenolic compounds and phycobiliproteins content, as well as its antioxidant capability along one year (from October 2018 to September 2019) and 2) determine the carrageenan features in one spot to establish seasonal differences and evaluate their rheological properties as a gelling agent to develop potential gelled matrices for commercial applications. The obtained data on dynamics of *M. stellatus* and the responses to environmental changes in different spots, offer a better understanding of their ecological behavior for a more effective management and sustainability for their economic exploitation.

Materials and methods

Study area

Figure 1 shows the sampling region of the red seaweed *Mastocarpus stellatus,* which was conducted on three rocky shores located in the North Coastline in Portugal, separated by approximately 13 km and selected for their easy accessibility.

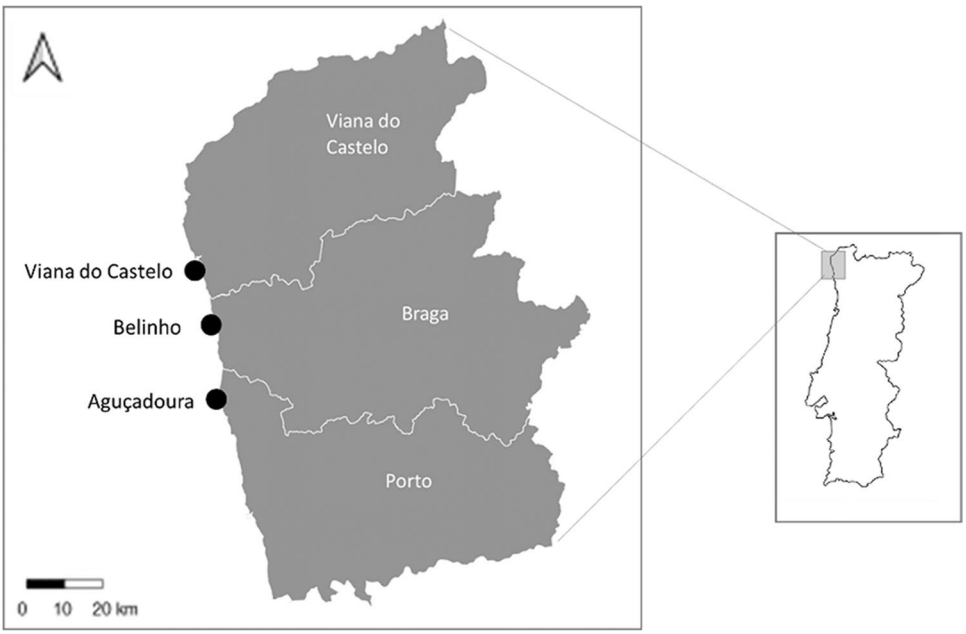

Fig. 1 Map showing the three sampling zones of the red algae *Mastocarpus stellatus*

Spot 1: located at Aguçadoura (41.432145°N, 8.784343°W) is a wide and extensive beach, located on Póvoa de Varzim. This shore is notable for its high exposure to waves and tides, a wave farm is placed nearby in the high seas converting wave and tidal energy into electricity. The beach is surrounding by anthropogenic pressure, such as urbanization and farming and is influenced by the Ave and Cávado river fluvial inputs. Spot 2: located at Belinho (41.590399°N, 8.804763°W), belongs to the protected landscape of the seashore of Esposende. The beach is characterized by an extensive rocky platform with a complexed dune system. The intertidal and low-shore zone are rich in variety of algae species, such as *Ulva* spp., *Chondracanthus acicularis*, *Osmundea pinnatifida*, *M. stellatus*, *Codium* spp. and *Chondrus crispu*s. And spot 3: Placed at Viana do Castelo (41.697601°N, 8.851618°W), is located across the mouth of the river Lima from the northern city of Viana do Castelo. The place consists of a rocky urban beach with elevate demographic pressure, sheltered with high biodiversity of seaweeds.

Seasonal climatic conditions

The climatological information of precipitation and temperature from October 2018 to September 2019 were obtained from the data series of the national meteorological stations published in the IPMA (The Portuguese Institute for Sea and Atmosphere) web site (www.ipma.pt/en/oclima/series.longas/). Solar radiation data were obtained from a 30-year average (1971–2000) historical model published in the Portuguese Official Climate Portal website (www.portaldoclima.pt).

Seaweed samples

The studied samples were collected in one-year campaign from October 2018 to September 2019 to evaluate their seasonal and spatial variations. Approximately 100 g of fresh seaweed were collected monthly in each spot, coinciding with the neap tides. Note that some data are missing due to unavailability of samples at the time of harvest. To prepare the samples, the fresh seaweeds were cleaned to remove salts, adhering sand and small shells, placed on metal trays and air convective dried at 60 °C for 48 h. Finally, dehydrated samples were ground to a particle size < 0.5 mm by a food processor (Thermomix, Germany) for better storage and handling. Micronized samples were stored in plastic bags, avoiding contact with light.

Biochemical characterization

Lipids quantification

A gravimetric method was used to determine the percentage of lipids adapted to seaweed biomass (Folch et al., 1957). One gram of micronized seaweed was added to 5 mL of a mixture of chloroform:methanol (ratio 1:1, v/v) in a homogenizer (Precellys Evolution, Bertin instruments, France). The mixture was placed in a 15 mL lysing tube using six ceramic (zirconium oxide) beads with 6 min cycle at 1075 G-force units (30 s homogenization with 40 s of stopping intervals). Subsequently, KCl (2 mL, 0.88%) was added and the mixture centrifuged for 5 min at 40 G-force units. The liquid phase was filtered and evaporated in a rotavapor. The analyses were performed in triplicate and expressed as percentage of dry weight (% DW).

Ash content

The ash content was determined by a standard gravimetric method, using a muffle furnace (Nabertherm B170, Germany) at 550 °C for 4 h. Quantification was made in triplicate and expressed as percentage of dry weight.

Carbohydrates quantification

The determination of carbohydrates was conducted spectrophotometrically using a calibration curve of glucose as standard. Distilled water (0.5 mL) and dry biomass (2.5 mg) were homogenized (Precellys, Bertin Instruments) as described in the previous section. Sulphuric acid (2 mL, 1 M) was added and incubated for 1 h at 100 °C in a laboratory oven. The mixture was cooled to room temperature and centrifuged at $161 \times g$ for 15 min. Then, distilled water (300 µL), phenol (400 µL, 5%) and concentrate sulphuric acid (2 mL) were added in an ice bath to 100 µL supernatant. After the exothermic reaction, the samples were left in hot water (25–30 °C) for 30 min. The absorbance was measured in triplicate at 490 nm. The results were expressed as percentage of dry weight and analyses were performed in triplicate.

Total phenolic quantification

Total phenolic content was determined in triplicate by the Folin-Ciocalteu method (Folin and Ciocalteu 1927). Dry seaweed (10 mg) and methanol (1 mL, 80%) were agitated in a homogenizer (Precellys, Bertin Instruments) as previously detailed. Then, 25 µL of extract was mixed with 125 µL distilled water, 25 µL Folin-Ciocalteu reagent and 75 µL sodium carbonate solution (6.6% w/v) and kept for 1 h in darkness, at room temperature. The absorbance was read at 760 nm, being the analyses were made in triplicate and the results reported as percentage of dry weight.

Total phycobiliprotein quantifications

Total phycobiliproteins were determined from the pigment absorption spectrum, according to the method described by Bennett and Bogorad (1973) and adapted for red seaweed based on Kursar et al. (1983). Dry biomass (5 mg) was mixed with 1 mL of 50 mM phosphate buffer, pH 7 at the same conditions as described in the previous section in a homogenizer (Precellys, Bertin Instruments), followed by 5 min of centrifugation at 161 × *g*. The absorbance from the liquid supernatant was determined by a UV/VIS spectrometer (Thermo Scientific Multiskan GO) on a microplate base at the wavelengths of 498.5, 614 and 651 nm following the procedures described for red seaweed (Kursar et al. 1983; Rubi et al. 2019). The total phycobiliprotein quantification of each sample was the sum of phycocyanin, allophycocyanin and phycoerythrin expressed as percentage of dry weight was calculated as phycocyanin = (151.1 A_{614}) – (99.1 A_{651}); allophycocyanin = (181.3 A_{651}) – (22 A_{614}) and phycoerythrin = (155.8 A_{498}) – (40 A_{614}) – (10.5 A_{651}). The results were obtained in triplicate.

Antioxidant capacity assessment

The ABTS assay, based on Guedes et al. (2013), was used to determine the total antioxidant activity of *M. stellatus*, using Trolox as standard. Firstly, 35 mg of dried biomass and 5 mL distilled water were homogenized (Precellys, Bertin Instruments) as described in previous sections. An aliquot of 63 µL was added to 180 µL ABTS + (2,2´-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) solution and rested at room temperature for 6 min, before measuring the absorbance in a microplate UV/VIS spectrometer at 734 nm. The above results were obtained in triplicate and expressed as mg Trolox equivalent antioxidant capacity (TEAC) g_{DW}^{-1}.

Carrageenan properties

Extraction and yield The carrageenan extraction from selected samples was carried out at least in triplicate using a set of optimized parameters discussed in a previous study (Torres et al. 2016). The sample (Aguçadoura_August/19) was analyzed to determinate the carrageenan content. The result obtained was compared with that collected in autumn (Aguçadoura_ October/18) to study differences according to seasonality in the target spot. Note that no comparative studies were conducted in the rest of the beaches. Briefly, seaweed powders (1.5 g) were dispersed in 100 mL of distilled water at 90 °C for 2 h. The corresponding suspensions were cooled (to 50 °C) and treated with α-amylase (1 mg g^{-1} algal material) (Fluka Chemie AG, Switzerland) for 1 h to digest floridean starch. Afterwards, the samples were heated (up to 80 °C) to favor centrifugation (161 × *g*, 15 min). The supernatants were precipitated in ethanol (2.5 volumes) and precipitates were vacuum filtered and oven dried (40 °C, 24 h). Note here that the carrageenan yield was determined using a conventional gravimetric method.

Fourier Transformation Infrared Attenuated Total Reflectance

To qualitatively analyze the carrageenan fractions, representative samples were studied. Fourier Transformation Infrared Attenuated Total Reflectance (FTIR-ATR) measurements were made from 500 to 1500 cm^{-1} at room temperature using a spectrometer (Nicolet 6700) as previously described by Pereira et al. (2009). Tests were performed at least in duplicate.

High-Performance Size-Exclusion Chromatography

To estimate the carrageenan molar mass distribution from the above representative samples, high-performance size-exclusion chromatography (HPSEC) measurements were run at least in duplicate. An arrangement of two columns in series (6 × 150 mm TSKGel SuperMultipore PW-H from Tosoh Bioscience, Germany) fitted by a TSKGel guard column SuperMP (PW)-H (4.6 × 35 mm) and a refractive index detector was employed. The used mobile phase was Milli-Q water at 0.6 mL min^{-1}. Poly(ethylene oxide) with different molecular weight (2.36 × 104 – 7.86 × 10^5 g mol^{-1}) (Tosoh Bioscience, Japan) were employed as standards. Measurements were made at least in triplicate.

Rheology

Aqueous solutions of carrageenan were made at commonly used biopolymer (1.0% w/w) and ionic (0.1 molar, KCl) content, by dissolving the appropriate biopolymer amount in the corresponding salt solution at 80 °C for 1 h under strong stirring to ensure full dissolution of the carrageenan. After formulation, samples were cold stored (4 °C) for 24 h before rheological testing. Small amplitude oscillatory shear measurements were made, at least in duplicate, on the formulated biopolymer gelled matrices using a MCR302 controlled-stress rheometer (Anton Paar Physica, Austria). A sand blasted parallel plate (2 mm gap, 25 mm diameter) was used to follow the viscoelastic behavior, in terms of elastic (G') and viscous (G'') moduli, of the systems, at 25 ºC. The samples were carefully loaded on the measuring geometry, sealed with light paraffin oil, and left to rest for 5 min. Then, stress sweeps were run at 1 Hz to define the linear viscoelastic region (< 35 Pa). Subsequently, frequency sweeps at 15 Pa from 0.1 to 10 Hz were conducted to monitor the viscoelastic behavior of the gelled matrices.

Statistics

Experimental data are expressed as mean ± standard deviation, with n = 3 the number of independent samples used to perform the statistical analysis. Data were statistically assessed using a one-factor analysis of variance (ANOVA) employing Minitab 20.4.0 software. A post-hoc Scheffé test was performed whenever differences between means (95% confidence, $p < 0.05$) of the monthly composition of each parameter for the global set of locations were found.

Results

Climatic conditions

The North-West region where *Mastocarpus stellatus* was collected showed a clear difference in temperature and rainfall during the months of study (Fig. 2). The maximum amount of precipitation (178 mm) and the minimum temperatures (below 10 °C) was achieved in winter, whereas the months of precipitation drastically decreased during the summer and temperature increased, reaching values above 20 °C. During the spring, the precipitation reached values of 50–100 mm and average temperature of 14 °C. The average precipitation and temperature of the studied period coincided with those obtained in the 30-year time series, classifying this region as temperate continental characterized with dry and mild summers. The incidence of solar radiation obtained from long time series (Fig. 2b) showed maximum values for the months of June-July (with 254–264 W m^{-2}) and minimum values of 57 W m^{-2} for December.

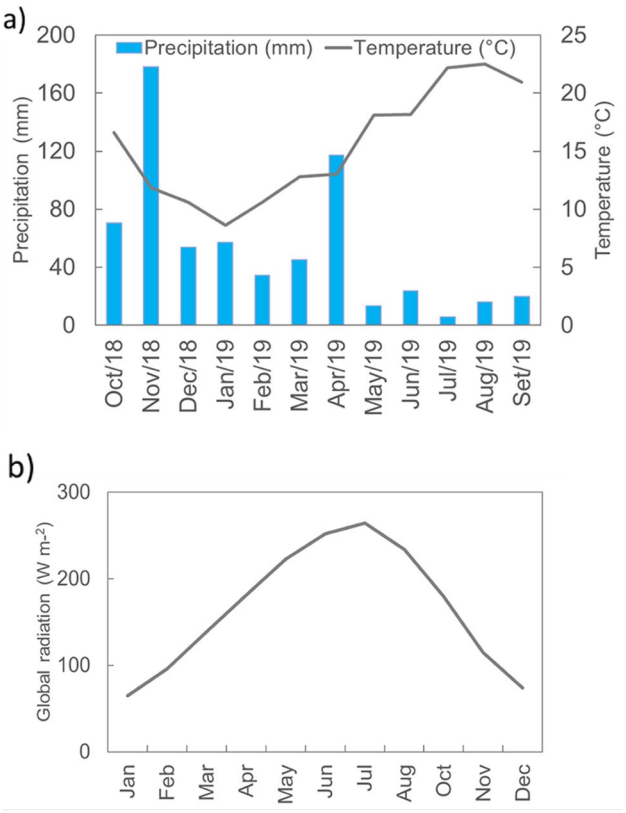

Fig. 2 a) Average values of temperature (°C) and precipitation (mm) in the Northeast region of Portugal between October 2018 to September 2019. Data obtained from www.ipma.pt. b) Global radiation values expressed in W m^{-2} in the period of (1971–2000). Data obtained from www.portalclima.pt

Biochemical characterization

Figures 3–9 show the annual variation of the biochemical composition of *M. stellatus* in terms of lipids, ash, carbohydrates, phenolic compounds and phycobiliproteins determination in the three studied locations.

The lipid content ranged from 0.4 – 2% DW in the three sampling locations (Fig. 3). There were statistical differences (F: 10.2, n: 3, $p \leq 0.05$) in the lipid content throughout the study year. Viana do Castelo exhibited the highest significant differences in May (F:9.3, n:3, $p \leq 0.05$) when compared to the annual lipids amount for the whole data sets. No notable differences were identified for the lipid profiles between Aguçadoura and Belinho sites.

The ash content for *M. stellatus* varied between 7 and 23% DW depending on the month and location (Fig. 4). A clear temporal variability between months was observed, where the ash concentration starts to increase in spring (from March–May), reaching its maximum percentages in summer (August – September). No statistical spatial significance was observed between harvest spots (F: 25.2, n: 3, $p \leq 0.05$).

Results showed that the carbohydrates content of *M. stellatus* samples varied between 2–36% DW (Fig. 5). Significant differences (F: 18.3, n: 3, $p \leq 0.05$) in the carbohydrate content were observed throughout the months studied. The highest values of this parameter were found in January in Belinho. The carbohydrate variations were more drastic in Belinho beach, reaching a maximum value of 36% DW in January compared to the maximum values at Aguçadoura (20% DW, February) and Viana do Castelo (25% DW, November). In general, cold months (from October to March/April) involved the highest carbohydrate content in Aguçadora and Belinho, decreasing significantly (F: 9.5, n: 3, $p \leq 0.05$) between May and July.

The carrageenan content of *M. stellatus* showed a seasonal variability of 7.5–22.1% during the studied period (Fig. 6). Significant differences (F: 4.5, n: 3, $p \leq 0.05$) were observed in the tested period. Aguçadora and Belinho showed similar profiles, with an increase in carrageenan content from October to August. The highest carrageenan

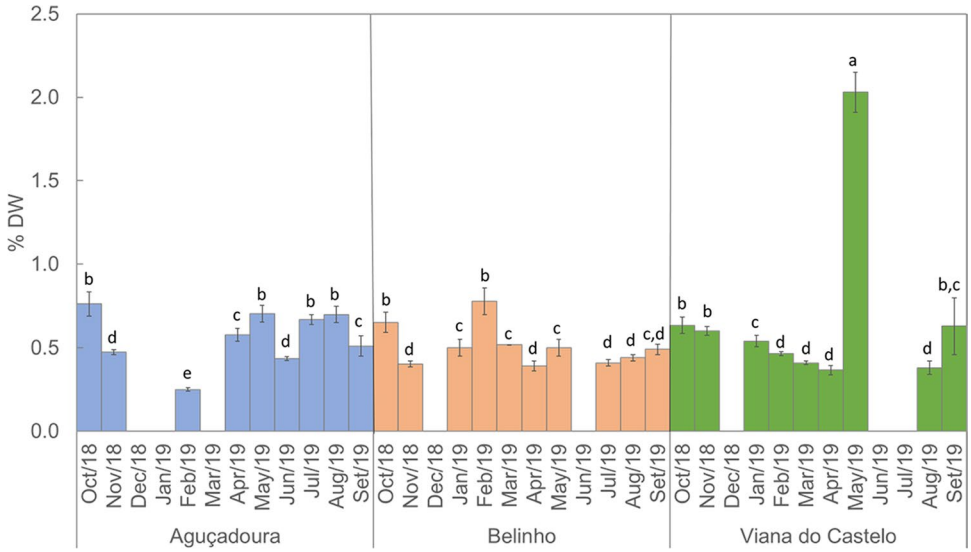

Fig. 3 Lipid content for *Mastocarpus stellatus* expressed as percentage of dry weight (DW). Letters above the bars indicates significant differences within the months with of 95% confidence, $p < 0.05$. Error bars indicate the standard deviation for $n = 3$

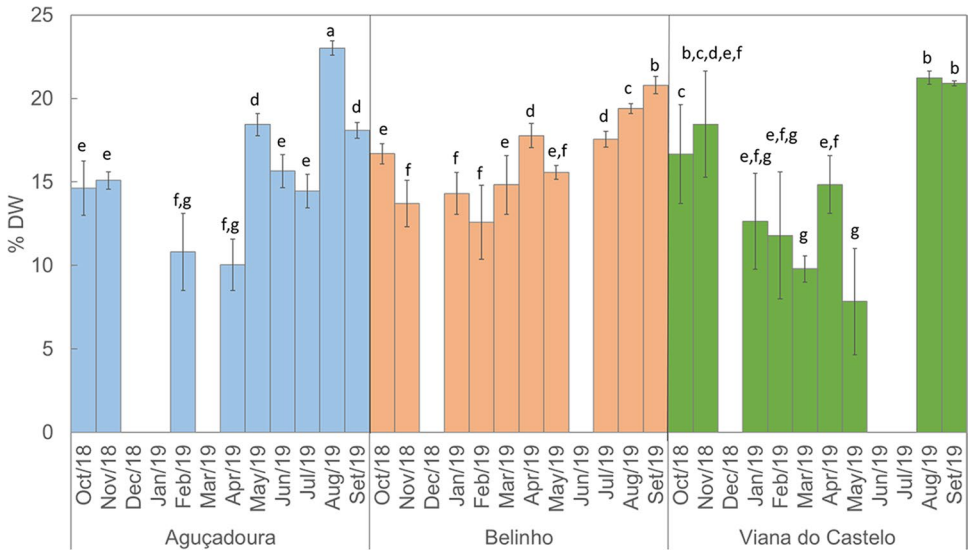

Fig. 4 Ash content for *Mastocarpus stellatus* expressed as percentage of dry weight (DW). Letters above the bars indicate significant differences within the months with of 95% confidence, $p < 0.05$. Error bars indicate the standard deviation for $n = 3$

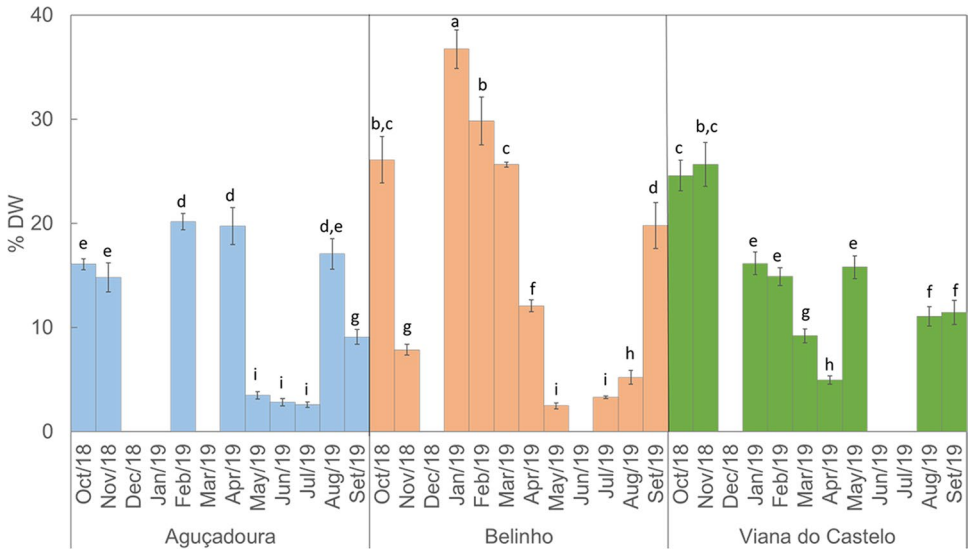

Fig. 5 Carbohydrate content for *Mastocarpus stellatus* expressed as percentage of dry weight (DW). Letters above the bars indicate statistical differences within the months with of 95% confidence, $p < 0.05$. Error bars display the standard deviation for $n = 3$

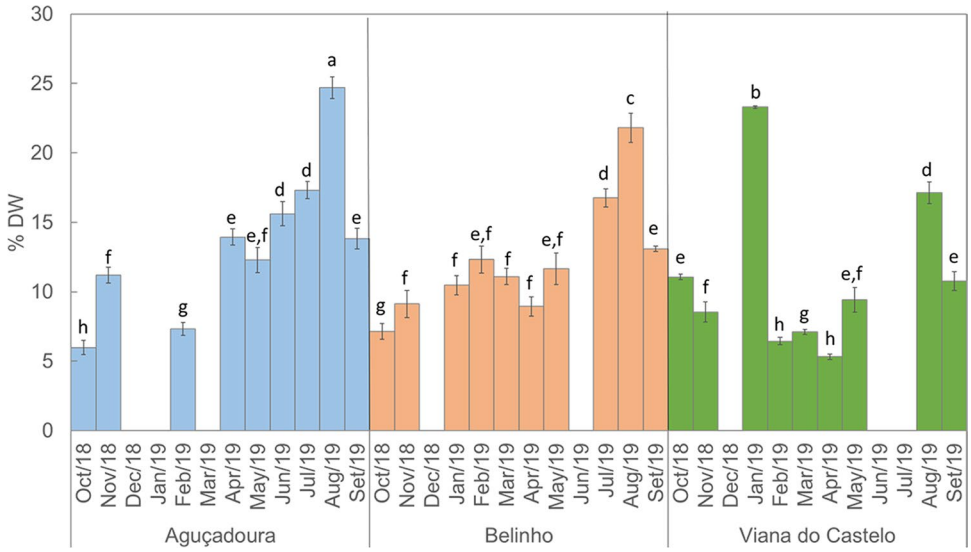

Fig. 6 Carrageenan content for *Mastocarpus stellatus* expressed as percentage of dry weight (DW). Letters above the bars indicate significant differences values within the months with of 95% confidence, $p < 0.05$. Error bars display the standard deviation for n = 3

content was identified in Aguçadora in August, followed by Viana do Castelo in January and Belinho in August.

Figure 7 displays representative FTIR spectra profiles. The selected samples featured typical *kappa/iota-* hybrid carrageenan profiles, with bands associated with ester sulfate bonds (950–700 cm^{-1}); 3,6-anhydro-galactose (930 cm^{-1}) characteristic of *kappa*-carrageenan; *δ*-galactose-4-sulfate (845 cm^{-1}) linked to both *kappa-* and *iota*-carrageenan; and 3,6-anhydro-galactose-2-sulfate (805 cm^{-1}) characteristic of *iota*-carrageenan. Note here that extracted hybrid carrageenans had average molecular weights (g mol^{-1}) of about 3.2 10^4 (Aguçadoura_October/18) to 5.4 10^5 (Aguçadoura_August/19), which were estimated based on empirical equations reported for molecular weight determination from corresponding HPSEC profiles for other similar carrageenophyte seaweeds.

The viscoelastic behavior of the hybrid carrageenans formulated in potassium chloride exhibited characteristic gel profiles, with elastic modulus (G') > viscous modulus (G'') (about tenfold) and almost frequency invariant (Fig. 10). Hybrid carrageenan extracted from Aguçadoura_August/19 samples led to stronger gels (i.e. higher viscoelastic moduli at fixed frequency) than those made with Aguçadoura_October/18 samples. It should be remarked that no water

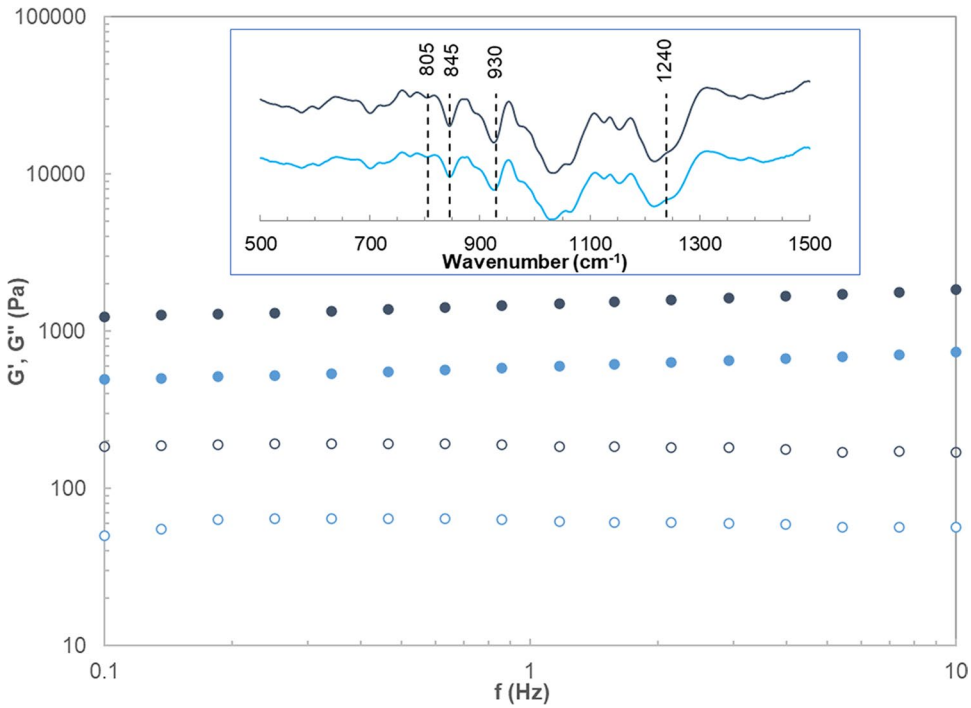

Fig. 7 Rheological profiles for representative carrageenans extracted from *Mastocarpus stellatus* red seaweed. Inset corresponds to the FTIR spectra associated to the plotted biopolymers. Symbols: G' (closed), G'' (open), Aguçadoura_August/19 (dark blue) and Aguçadoura_ October/18 (light blue). No error bars were included when lower than the symbol size

syneresis was identified after one week of cold-storage of the biopolymer- based gelled matrices.

Total phenolic content was lower than 0.20% DW in all tested samples (Fig. 8). Aguçadoura and Viana do Castelo exhibited higher values than those found in Belinho beach. Significant annual variations (F: 10.5, n: 3, $p \leq 0.05$) were identified for this parameter. Total phenolic content showed the maximum values between February and May and again between October and November in the three harvested spots.

Figure 9 represents the phycobiliprotein content, expressed as the sum of the phycocyanin, allophycocyanin and phycoerythrin amount in each sample. The magnitude of these proteins varied between 1.4 to 3.0% (dry weight) for. The highest content of phycobiliproteins was concentrated in October/November in the three studied locations. Aguçadoura was the site where the best phycobiliprotein results were obtained, since a second maximum peak of pigment was observed in April. No statistical differences (F: 3.4, n: 3, $p < 0.05$) were found between the rest of the months. Likewise, no significant differences (F: 5.2, n: 3, $p < 0.05$) were found between the different locations.

Figure 10 shows the total antioxidant activity determined with the ABTS method. Results varied in a range of 2—4.4 mg $_{Trolox}$ mg_{DW}^{-1} depending on season and location. Significant annual variations (F: 9.3, n: 3, $p \leq 0.05$) were found for this parameter. The graphics showed a progressive increase from October to January/ February and a progressive decrease from February to September. The highest

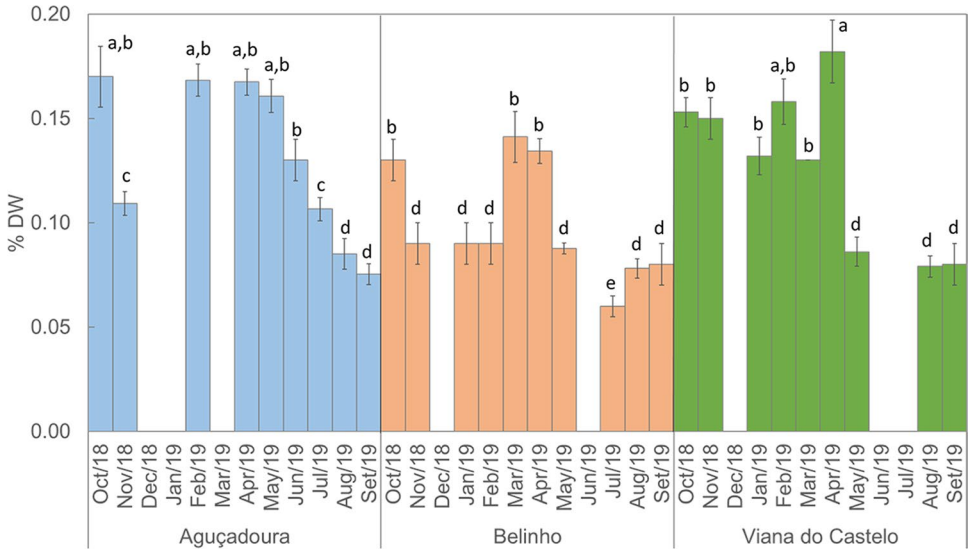

Fig. 8 Total Phenolic Compounds for *Mastocarpus stellatus* expressed as percentage of dry weight (DW). Letters above the bars indicate statistical differences within the months with of 95% confidence, $p < 0.05$. Error bars show the standard deviation for n = 3

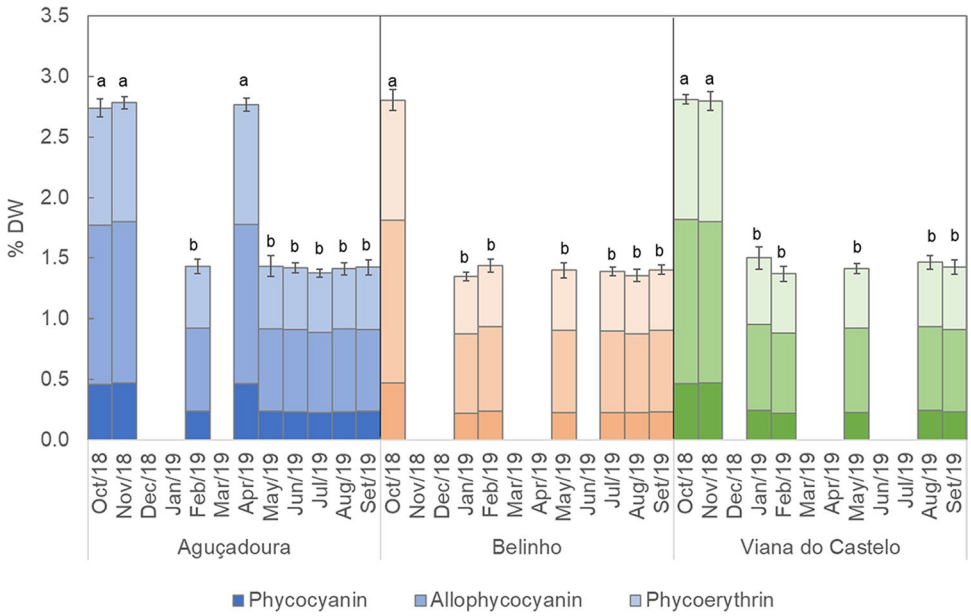

Fig. 9 Total phycobiliproteins for *Mastocarpus stellatus* expressed as percentage of dry weight (DW). Letters above the bars indicate significant differences between the months with of 95% confidence, $p < 0.05$. Error bars display the standard deviation for n = 3

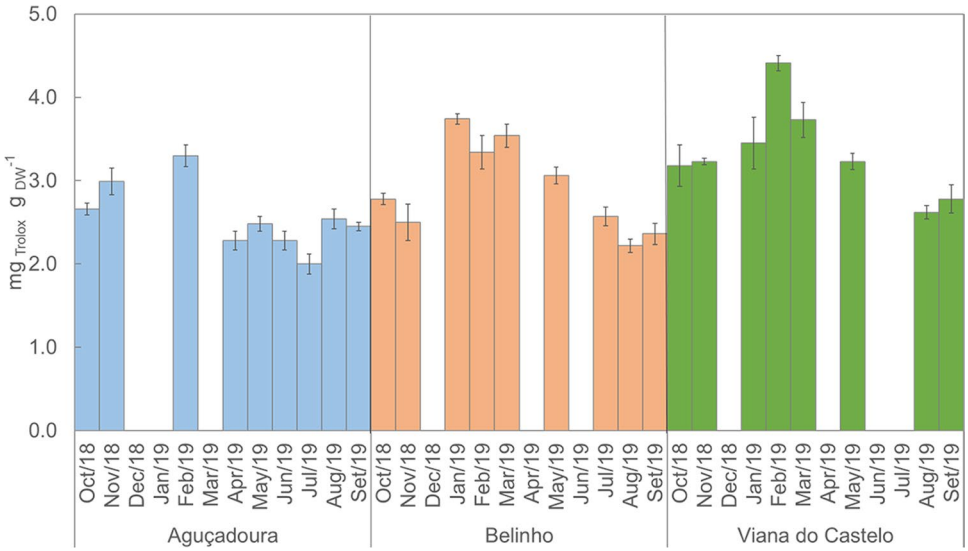

Fig. 10 Antioxidant activity for *Mastocarpus stellatus* expressed as percentage of dry weight (DW). Letters above the bars indicate differences within the months with of 95% confidence, $p < 0.05$. Error bars indicate the standard deviation for $n = 3$

antioxidant activity was observed in February for the three sampling locations, which is consistent with the outcomes previously achieved for total phenolic content.

Discussion

Seaweed composition suffers different changes caused by the location, environmental conditions, life cycle, growth, among other factors (Cherry et al. 2019). The determination the cause of their variability is decisive to identify the proper harvest moment when the species is most productive for the particular use in the industry. The biocompounds of *M. stellatus*, showed significant seasonal and spatial differences in the studied period.

The low lipid content observed throughout the study is ideal for the introduction of *M. stellatus* in the human diet, also considering its richness in polyunsaturated fatty acids (PUFAs) (Koch et al. 2017), which in recent years has raised commercially interest because their applications in the prevention of heart diseases, diabetes and tumor treatments (Kendel et al. 2015). This red seaweed could be considered as a promising natural source of healthy fatty acids. The obtained results are consistent with previous studies (Maghraby and Fakhry 2015; Yang et al. 2021), where studied macroalgae reached the highest lipid content in spring/summer, attributed to the warmest temperature of the sea water. The influence of seasons on the fatty acid content of different macroalgae was also reported by Pereira et al. (2021), who found that the fatty acid production was promoted in two brown seaweeds, *Fucus spiralis* and *Bifurcaria bifurcata*, during spring and summer, whereas the same was verified in winter for *Ulva lactuca* green seaweed.

The ash content in seaweeds is believed to depend on intrinsic and external factors and is attributed to the type and content of biopolymers in the algae cell wall, and their own ability to absorb the substances from the environment (Biancarosa et al. 2017; Lozano Muñoz and Díaz 2020). Among external factors, environmental seasonality has an influence on the ash content and minerals profile of the red seaweed, doubling the concentration from summer to the winter season (Circuncisão et al. 2018). The high values of ash detected in the seaweeds in this study are consistent with other studies from edible seaweed (Sánchez-Machado et al. 2004; Yang et al. 2021). Latter authors reported ash values of 28.17% from fresh *M. stellatus* and 19–34 g $(100\ g)^{-1}$ dry weight from commercial canned seaweeds. It should be highlighted that ash content of the traditional vegetables is lower than those found in macroalgae, only the higher values observed in spinach (20% dry weight) are comparable to those of seaweeds (Cardoso et al. 2014; Circuncisão et al. 2018). It was also observed that the ash content of different red seaweeds like *Grateloupia doryphora* was positively correlated to protein content and inversely to the carbohydrate content (Shabaka and Moawad 2021).

Carbohydrates are the most abundant constituents of seaweeds and varies seasonally due to changes in nutrient availability during the year (Kaehler and Kennish 1996). The results of the carbohydrate content analyzed indicated that in seasonal terms, the highest concentrations of carbohydrates were found in the cold months (from October to March/April), coinciding with the raining season and at the time when there is a greater contribution of nutrients and water from the surrounding rivers. The carbohydrate concentration decreased in the warm season, coinciding with periods of drought. Studies from seasonal variations of red seaweeds have shown that the extremely high surface water temperature and high salinity concentrations during warm periods result in desiccation of seaweed and subsequent reduction of carbohydrate content (Banerjee et al. 2009).

The determination of the carrageenan content has a relevant importance in the study, due to *M. stellatus* is characterized by their high content of carrageenan (Kim et al. 2018; Ruocco et al. 2016). Seasonality influences the carrageenan profile of the seaweed, being the results consistent with the studies from Tasende et al. (2013) about seasonal quantification of carrageenan content from *M. stellatus* seaweeds in the North-West of Spain, where the maximum content of carrageenan was observed in August, coinciding in the periods in which the solar incidence and the temperature of the water is higher. The incrementation content of carrageenan in summer is also related to the life cycle of the seaweed, being April-June the growth period of this species, resulting in a greater accumulation of carrageenan in this period of the year (Yang et al. 2021). In this study case, Viana do Castelo showed an unusual point reached in January, this phenomenon could be caused by the adaptation of algae to environmental and oceanographic punctual changes.

Mastocarpus stellatus grows in the intertidal zone and has to survive in a competitive and constant changing environment with high light and oxygen fluctuations, so that, they developed protective antioxidant defense system composed from different metabolites, such as phenols (Jiménez-Escrig et al. 2012), considered one of the most powerful antioxidants in seaweed. The maximum values of phenolic compounds achieved in the study, coincide after the raining season when the nitrogen concentration in water raised due to the contributions of nutrients from fluvial waters from the nearby rivers. Previous studies demonstrated that a higher nutrient content diminished the quantity of phenolic compounds because the seaweed is focusing on growth (Lomartire et al. 2021). Thus variability in the phenolic quantity throughout the year, is also a cellular defensive response and serves to prevent and protect seaweed from the attack of bacteria, microalgae, fungi, invertebrates and survive in those stressful conditions (Wikstrom and Pavia 2004). The total phenolic content can be also affected by the drying process (Ling et al. 2015; Uribe et al. 2020). Ling et al. (2015) pointed that samples of seaweed dried by oven drying between 40–80 °C, showed higher values of total phenolic compounds than those dried by the sun-drying techniques exposed under direct sunlight for 3–4 days. The extraction of this bioactive from seaweed could be used in potential industrial activities and applications developing novel products such as natural and non-harmful food stabilizer, as well as skin care and anti-aging cosmetic products (Cotas et al. 2020; López-Hortas et al. 2018, 2021).

In marine environments, the solar irradiance and the spectral distribution of light suffer some phenomena such as reflection, dispersion, and absorption different than land environment. Marine organism such as red algae can develop auxiliary photosynthetic complexes that allow them to live in environments with variable solar irradiation (Jorge Dagnino-Leone et al. 2022). The phycobiliproteins, present in red algae, absorb solar radiation which is then passed on to chlorophylls during photosynthesis. The phycobiliprotein content showed both spatial and season variability in the study period. The highest concentration of phycobiliproteins were observed in October/November. During the summer, the algae decrease the pigment production because the abundance of sun light available, affecting the photosynthetic levels and reaching minimum values, so that the seaweed produces more pigments in the winter since it needs to optimize the sunlight capture for surviving (Pereira et al. 2012). These biocompounds could be used as colorants, fluorescent markers, antioxidant, anti-inflammatory, disease inhibitor among others for commercial applications (Li et al. 2019).

Total antioxidant activities determined in this work followed the same trend as those observed in the quantification of phenolic compounds, where the total antioxidant capability reaching the lowest antioxidant capacity in summer and the maximum in late winter / early spring, this could be caused by tolerance to environmental stress, particularly in winter. Photosynthesis activities increased in winter when air and water remain cold and the sun irradiance is lower, as well as the contribution of light is scarcer. Studies from Lohrmann et al. (2004) of seasonal acclimatation of antioxidants in *M. stellatus* to different stress conditions, confirm that antioxidants increases when the air and water temperature remains below 7.5 °C in the cold months (December–April) than in the warm months when the temperature is above 11 °C (June – October) indicating that antioxidant capacity increases in winter due to acclimatization. The extraction of antioxidants from marine resources using environmental friendly technologies is gaining more relevance in recent years (Ponthier et al. 2020; Flórez-Fernández et al. 2022), where liquid fractions rich in antioxidants are incorporated into gelling matrices to produce functional hydrogels (Ponthier et al. 2020; Rodríguez-Seoane et al. 2021; Sanz et al. 2021).

The carrageenan properties extracted from *M. stellatus* collected in Aguçadoura beach between October/18 and August/19 and the corresponding viscoelastic behavior were successfully studied. The carrageenan yields were consistent with those previously reported using conventional alkali extractions for similar carrageenophyte seaweeds (Azevedo et al. 2015), showing typical *kappa/iota* hybrid carrageenan profiles. The rheology studies performed, exhibited a typical gel profile (elastic modulus (G') > viscous modulus (G")) almost invariant over the tested frequency range (Moreira et al. 2011). Both samples showed intermediate gel strength, with viscoelastic properties in the range of those reported for hybrid carrageenans extracted from *M. stellatus* using conventional (Azevedo et al. 2014) or microwave extraction (Ponthier et al. 2020) procedures. Hybrid carrageenan from Aguçadoura_August/19 showed higher viscoelastic moduli

than those from Aguçadoura_October/18, displaying intermediate gel strength features. The recovery of hybrid carrageenans with different viscoelastic properties would allow the formulation of gelled matrices of different strength, and therefore suitable for different applications. In this field, there are studies on the application of hybrid *kappa/iota* carrageenan and their properties as antimicrobial, antivirals, tissue repair, antifungal or anticancer molecules where seaweed such as *Chondrus*, *Mastocarpus* or *Anhfeltiopsis* produce hybrid carrageenans with these types of properties, sometimes linked with other carrageenan fractions (Soares et al. 2016, 2021; Ramezanpour et al. 2017; Souza et al. 2018; Calvo et al. 2019; Pettinelli et al. 2020; Frediansyah 2021).

Conclusion

To conclude, wild *Mastocarpus stellatus* collected in the North-West of Portugal presented significant annual variability in the main components content highly dependent on the sampling locations. The months of higher solar incidence promoted the seaweed productivity, except for carbohydrates and phycobiliproteins. Viana do Castelo was more productive in antioxidants, Belinho in carbohydrates and Aguçadoura in kappa/iota- hybrid carrageenan. The structure and viscoelastic properties of the biopolymer were not jeopardized independently of the extraction season. Further studies about the interference of oceanographic, environmental, and human factors are needed to establish a correct management of this raw material as an alternative source of biocomponents for commercial proposes for the formulation of new products for the food and no food industry.

Authors' contributions A.C.G., I.C. and V.S. conceived and designed research. V.S. and M.D.T. performed the experimental tasks, V.S. and M.D.T. wrote a draft of the manuscript; M.D.T., H.D., I.S.P., I.C. and A.C.G. followed the experimental tasks, analyzed the results, and corrected the manuscript. A.C.G., I.S.P., M.D.T. and H.D were responsible for the supervision and funding acquirement. All authors read and approved the manuscript.

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Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests Authors declare there is no conflict of interest.

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