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Short communication

Sargassum filipendula alginate from Brazil: Seasonal influence and characteristics

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ABSTRACT

The aim of this work is focused on the extraction and characterization of the Brazilian seaweed *Sargassum filipendula* alginate. Alginates obtained at different seasons were characterized by liquid state nuclear magnetic resonance spectroscopy and scanning electron microscopy. The alginate extraction efficiency was about 20%. Different seasons of the year and different stages in the life cycle of *Sargassum* sp. in south-eastern Brazil influenced the M/G and, consequently, the technological properties of extracted alginates.

1. Introduction

Brown seaweed can be composed of up to 40% of its dry weight by alginic acid (Percival & Mcdowell, 1967), a natural polymer widely used in food industries, textiles, cosmetics and pharmaceuticals due to its gelling properties, viscosity and stability. Alginic acid has also a strong interaction with heavy metals, allowing its application as an alternative treatment for aqueous effluents.

Alginate is the common name given to a family of linear polysaccharides containing 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) acid residues arranged in a non-regular, block-wise order along a chain (Haug, Larsen, & Smidsrod, 1967; Hirst & Rees, 1965; Rees & Samuel, 1967). It has been clearly shown that the alginate physical properties depend not only on the uronic acid composition, but also on the relative M- and G-block sequences, which cause significant structural differences (Indergaard & Skjak-Braek, 1987; Indergaard, Skjak-Braek, & Jensen, 1990). M/G block relationship in the alginate structure has also an important role on the reactivity of these polysaccharides (Haug et al., 1967). The gelation of alginates is dependent on the number and length of the polyguluronate sequences (Rees, 1972).

The abundance, conformation and proportion between acid blocks depend not only on the species (Haug, Larsen, & Baardseth, 1969) but also on the season (South, 1979), age and source of the algae (Davis, Ramirez, Mucci, & Larsen, 2004; Davis, Volesky, & Mucci, 2003; Haug, Larsen, & Smidsrod, 1974; Indergaard & Skjak-Braek, 1987; Indergaard et al., 1990; Larsen, Salem, Sallan, Mishrikey, & Beltagy, 2003). These factors reflect the functional properties of alginate, solubility, interaction with metal ions, gelling and viscosity (Steginsky, Beale, Floss, & Mayer, 1992).

The alginate ability to form gels in the presence of divalent/trivalent cations such as calcium(II) is key to its biological functions and applications. When di- and tri-valent cations are present, the alginate solutions form a gel as a result of polymer interchain associations. Sequences of polyguluronate packed together with strongly coordinated cations in cavities between the chains cause the gelation of these polysaccharides (Grant, Morris, Rees, Smith, & Thom, 1992; Morris, Rees, Thorn, & Boyd, 1978).

Commercial alginates are produced mainly from *Laminaria hyperborea, Macrocystis pyrifera, Laminaria digitata, Ascophyllum nodosum, Laminaria japonica, Eclonia maxima, Lessonia nigrescens, Durvillea Antarctica*, and *Sargassum* sp., with estimated annual world production of dry weight algae of ~85,000 tons, from which ~23,000 tons of alginate are obtained (Draget & Taylor, 2011). Alginate used by the Brazilian industry is either from Asia or Europe, but the abundance and diversity of macroalgae distributed throughout the Brazilian coast (Fortes-Xavier, 2000; Mchugh, 2003) makes it possible to explore locally derived alginate in a commercial manner. This happened in India, where native *Sargassum* species are an alternative source for extracting industrial alginate (Thomas & Subbaramaiah, 1991). Thus, our work aims to characterize the Brazilian seaweed *Sargassum filipendula* alginate extracted during

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different seasons and determine the seasonal influence on properties such as alginate yield, structure and M/G ratio.

2. Materials and methods

S. filipendula seaweed was collected in the seashore in the State of São Paulo (Cigarras beach, Brazil), in three different seasons: fall (May 10th, 2009), spring (November 21st, 2010), and summer (February 22nd, 2011). Algae samples were immediately dried at 60 °C during 24 h.

2.1. Alginate extraction

Alginate extraction was performed according to McHugh's (1987) method. Dry algae were soaked in formaldehyde (0.4%, w/w) for 0.5 h, washed with distilled water and added to an acid solution (HCl, 0.1 mol L^{-1}) for 2 h. Formaldehyde and hydrochloric acid treatments removed the phenolic compounds and bleached the material.

Bleached samples were washed with distilled water and extracted for 5 h with a 2% (w/w) sodium carbonate solution at 60 °C. In the presence of sodium carbonate excess, alginic acid is converted into soluble sodium alginate (Davis et al., 2003). Alginate samples were separated from the crude extract by ethanol precipitation. Extraction yields were calculated by the ratio of initial seaweed mass and obtained dry alginate mass.

2.2. Characterization

In order to evaluate the properties of interest of Brazilian alginates, extracted samples were characterized by nuclear magnetic resonance spectroscopy in liquid state (^{1}H NMR) to assess the relationship between guluronic and mannuronic acid. A Bruker AVANCE III 600 MHz equipment was used. Samples were prepared by partial acid hydrolysis method (Davis et al., 2004) and ran at 90 °C (363.15 K) using D_2O as solvent, first without water peak suppression for reference, followed by the suppression of water. The morphology of samples was investigated by scanning electron microscope. These data were obtained by using LEO equipment, LEO 440i. Samples received gold topping, 2000× (algae) and 1000× (alginate) power was performed.

3. Results and discussion

Alginate extraction yields from seaweed *S. filipendula* collected during fall and spring were 17.0 ± 0.1% and 17.2 ± 0.3%, respectively, while algae collected during summer showed a lower amount of alginate, with a yield of 15.1 ± 0.1%. Percentages obtained are consistent with the range observed in brown algae (10–40%). Torres et al. (2007) obtained a yield of 16.9 ± 0.7%, similar to the results in this paper, from the seaweed *Sargassum vulgare* (Brazilian Northeast region).

The *S. filipendula* images in Fig. 1 show absence of vesicle floats, considered an indicative of larger hydrodynamic forces acting on algae from areas with strong wave action. Algae collected during the summer (February) have receptacles which are characteristic of their reproductive period. Summer algae showed a lower amount of alginate in the branches and stems (15.1%) and no alginate precipitation from receptacles. This fact was also noted by Zubia, Payri, and Deslandes (2008) in *Sargassum mangarevense* and *Turbinaria ornata* collected on the Northwest inner barrier reef in Tahiti (French Polynesia). Alginate yields in *Sargassum* are known to be affected by environmental factors and biological functions of alginic acid (such as prevention of desiccation, flexibility) (Aponet de Otaola, Diaz-Piferrer, & Graham, 1983).

There was no significant presence of algae in the region during the winter period (August) because *Sargassum* species from Southeastern Brazil are pseudo perennial. They live for more than a year, but some of their branches can break off and in some situations, this loss can be considerable. McCourt (1984) suggested that in cold temperature and subtropical regions, the development of vegetative population occurs during spring or summer, which agrees with the results here in of higher alginate yields obtained during spring. Gillespie and Critchley (1999) also observed a reduction of algae through the winter months and a peak during summer months. Schenkman (1989) observed maximum biomass in late spring (November, December) and minimum values during winter (July) for *Sargassum cymossum* in Praia Grande, Ubatuba (SP, Brazil). Although *S. cymossum* is abundant throughout the year, it loses branches from the end of summer until midwinter.

The May seaweed micrograph looks rougher, with the presence of diatom algae. These mostly unicellular algae were also present in seaweeds from November and February. For alginate micrographs, the characteristics are similar in the three different seasons regarding the fibrous aspect.

M/G data were calculated based on the peaks areas from liquid ^{1}H NMR spectra as these have been shown to be very accurate for the determination of alginate composition and block structure (Larsen et al., 2003; Panikkar and Brasch, 1996). Fig. 2 shows the alginates liquid ^{1}H NMR spectra and whose peak areas were used in our calculations. In order to calculate the glucuronic (G) and mannuronic acids (M) ratio, the peak area of guluronic acid anomeric proton (G-1) at 5.18 ppm (I), mannuronic acid anomeric proton (M-1) and the H-5 of alternating blocks (GM-5) overlapping at 4.74 ppm (II), and guluronic acid H-5 at 4.51 ppm (III) and equations proposed by Grasdalen (1983) (Eq. (1)) and the n parameter (Eq. (2)) were used. According to Grasdalen, Larsen, and Smidsrod (1979), n values < 1 represent abundance of homopolymeric blocks. Peak areas, calculated values of M/G ratio and n parameter are summarized in Table 1.

$$\mathrm{M/G} = \frac{(1 - F_G)}{F_G} \tag{1}$$

$$n = \frac{F_{GM}}{(F_M + F_G)} \tag{2}$$

Each sample presented a different M/G ratio, indicative of seasonality influence on the type of sugar incorporated. The heterogeneity of the obtained data from the same specie shows the influence of the environment on the composition of alginates (Fenoradosoa et al., 2009). Authors such as Lin and Hassid (1966) and Madgwick, Haug, and Larsen (1973) suggest that the alginate can be initially biosynthesized as polymanuronic, with subsequent conversion to poliguluronic or mixed sequences by the enzymes actions at polymeric level. Polymanuronic acid can be transformed in polyguluronic acid by the enzyme C-5 epimerase (Currie & Turvey, 1982). This conversion offers a biological control mechanism for the tissue structure in response to the environmental changes (Larsen, 1981). A low M/G ratio (<1.00) indicates greater amount of G in the composition of alginates (Davis et al., 2003). Comparing the alginates obtained from *S. filipendula* collected in the Cigarras beach, the highest amount of G groups was detected in the February algae, followed by the algae collected during fall, while the algae in the end of spring presented lower amount of guluronic acid. The spring sample corresponds to a younger plant once *Sargassum* considerably loses its branches in winter and therefore, shows a higher amount of M blocks. The February algae presented higher amount of G groups even though it does not correspond to the oldest plant. This high amount of G groups can be related to the reproductive period of the algae. The values of $n < 1.0$ indicate abundance of homopolymeric blocks, which were confirmed for the

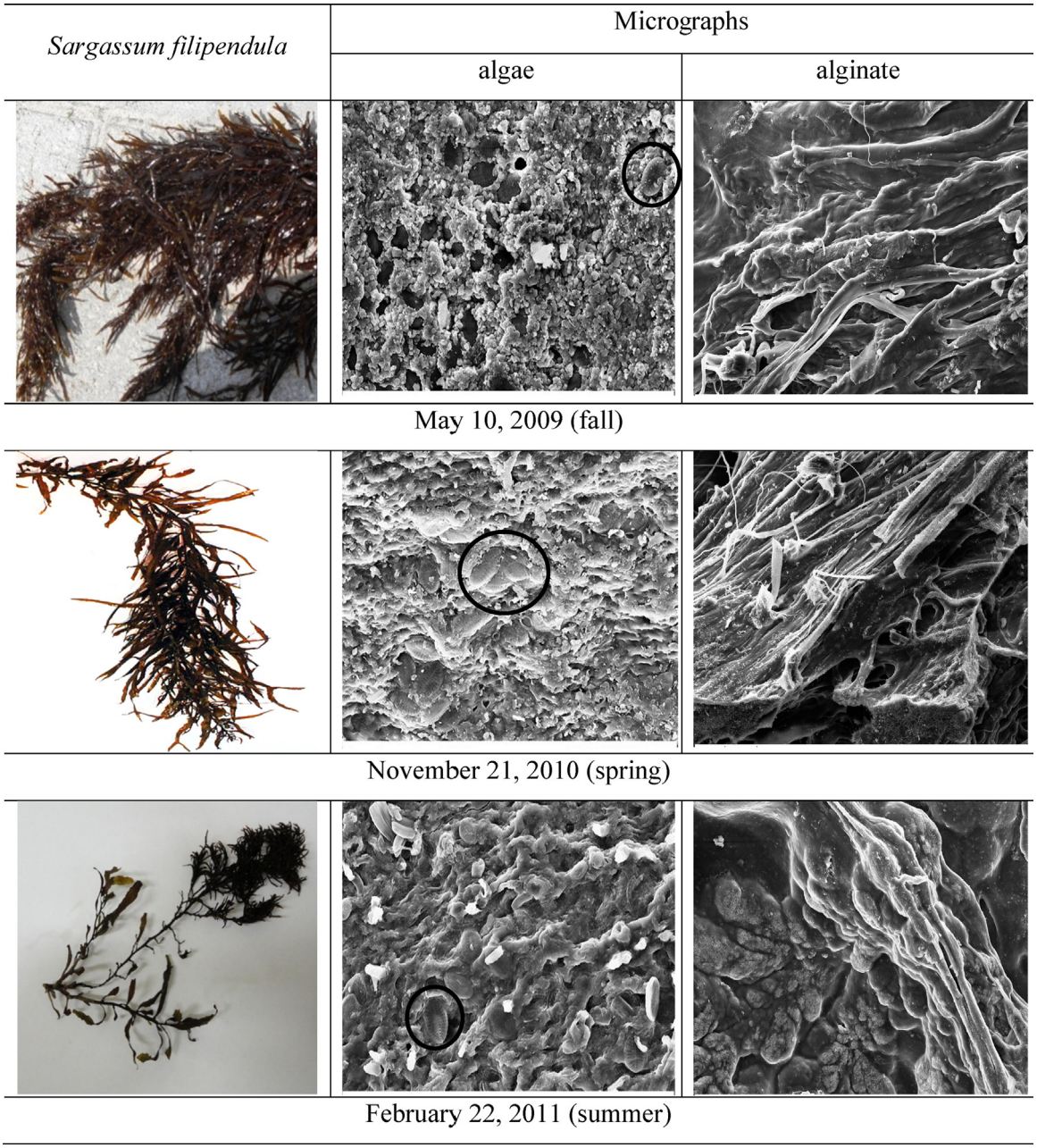

Fig. 1. *Sargassum filipendula* images (left), dry seaweeds (middle) and extracted alginates (right) micrographs. Circles indicate the presence of diatom algae.

samples being studied, with higher amounts of MM or GG blocks for the samples from May and November when compared to the February sample.

The M/G ratio is an important parameter that may imply on the biopolymer applications. Alginates with higher amounts of M presented low viscosity, are more flexible and useful to obtain polyelectrolyte complexes, for the production of micro- and nanoparticles of drugs, or for uses in paper industries. On the other side, alginates with greater viscosity and higher block G contents can be used to obtain more resistant gels and are applicable in food and cosmetic industries (Hernandez-Carmona, Mchugh, & Lopez-Gutierrez, 2000). If we compare the yield and M/G ratios of alginates extracted from the *Sargassum* species studied by other authors (Table 2), our results are in a good agreement and, except for the alginate isolated from *S. vulgare* by Torres et al. (2007), most alginates from the *Sargassum* species have a M/G ratio < 1.

In order to incentive the commercial exploitation of the alginate extracted from *S. filipendula* in Southeast Brazil, it is necessary

Table 1
Composition data of alginates extracted during different seasons of the year.

	I (area)	II (area)	III (area)	F_G	F_{GG}	F_M	M/G	n
Fall 2009 (May) (a)	1.00	0.31	0.26	0.57	0.46	0.43	0.75	0.11
Spring 2010 (November) (b)	1.00	0.31	0.25	0.56	0.45	0.44	0.78	0.11
Summer 2011 (February) (c)	1.00	0.35	0.25	0.60	0.42	0.40	0.67	0.18

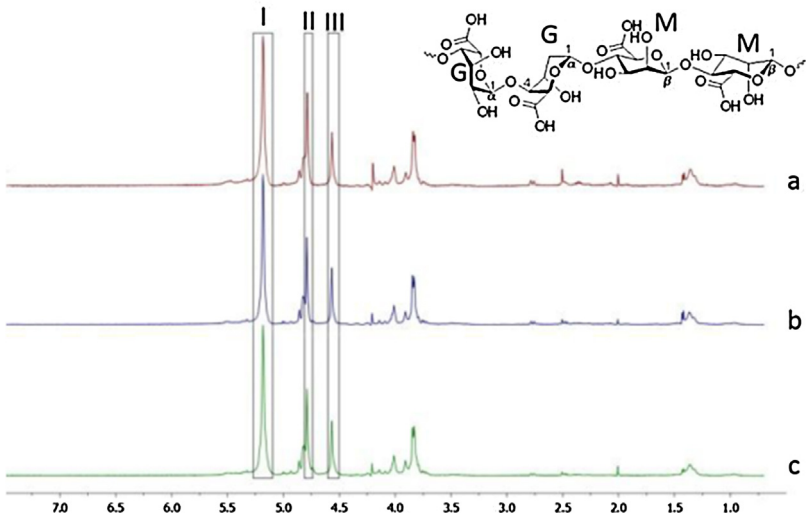

Fig. 2. Alginate ^{1}H NMR spectra in D_2O: (a) fall 2009 sample; (b) spring 2010 sample; (c) summer 2011 sample. Insert: Fragment of an alginate molecule showing two glucoronate units and two manuronic units.

Table 2
Yield (%) and M/G ratio from species of *Sargassum*.

Species	Yield (%)	M/G	Reference
S. vulgare	30.2	0.71	Behairy and El-Sayed (1983)
S. polycystum	17.1–27.6	0.56–0.74	Saraswathi, Babu, and Rengasamy (2003)
S. dentifolium	3.3	0.52	Larsen et al. (2003)
S. latifolium	17.7	0.82	
S. asperifolium	12.4	0.69	
S. oligocystum	16.3–20.5	0.49–0.62	Davis et al. (2004)
S. fluitans	21.1–24.5	0.52–0.57	
S. vulgare	16.9	1.27/1.56	Torres et al. (2007)
S. filipendula	15.1–17.2	0.67–0.78	This study

to have a more in-depth study, with monthly monitoring of the growth and density of the algae in the region, as well as its recovery after harvesting as to avoid environmental damages, and the determination of other biopolymer properties (viscosity and the molar mass).

4. Conclusions

Brazilian seaweed *S. filipendula* is composed from 15.1% to 17.2% in its dry weight by alginate, with algae spring samples showing to be the richest sources for this biopolymer extraction. A non-regular, block-wise order of β-D-mannuronic (M) and α-L-guluronic (G) acids varied along a chain in extracted alginates as a consequence of different seasons and stages in the life cycle of algae. It was observed that M/G ratios for all algae samples are below 1.0 and, consequently, the technological properties of the extracted alginates, with greater viscosity, are more appropriate for obtaining resistant gels for food and cosmetic industries application.

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