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Sulfated polysaccharides (fucoidan) from the brown seaweed *Silvetia compressa* (J. Agardh) E. Serrão, T.O. Cho, S.M. Boo & Brawley

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Abstract

The aim of this study was to characterize the yield and chemical compositions of sulfated polysaccharides (SP) from the brown seaweed *Silvetia compressa* collected off the Pacific coast of Baja California, Mexico. SP yield was evaluated from basal and apical tissues and fruiting bodies. Chemical heterogeneity was evaluated by ethanol fractionation and anion-exchange chromatography and characterized by FTIR spectra. Geographic differences in yield (11–12.1%) and sulfate content (15–16%) were minimal. Basal thallus had a higher yield, while fruiting bodies contained more fucose and sulfates. The SP of *S. compressa* are composed of variable amounts of fucose, sulfates, and uronic acids (fucoidan type). The heterogeneity of SP was demonstrated by fractionation with ethanol at 50% which yields a soluble fraction, composed of fucose with high sulfate content devoid of uronic acids. Similarly, the anion-exchange chromatography separated fractions composed of molecules differing in fucose content. FTIR spectra showed characteristic signals for SP: a strong peak at 1240–1250 cm^{-1} (S=O), a peak at 840–850 cm^{-1} (axial sulfate C-4), and peaks at 1625 and 1417 cm^{-1} (carboxylic group of uronic acids). These results indicate that SP of *S. compressa* correspond to a fucoidan-type polysaccharides with sulfates occurring mainly on C-4 of the fucose units and containing low amounts of uronic acids.

Keywords *Silvetia compressa* · Phaeophyceae · Fucoidan · Sulfated polysaccharides · Uronic acids

Introduction

The carbohydrates are one of the main components in marine seaweeds, representing nearly 50% of the dry weight. As in terrestrial plants, they act as energy storage and structural elements. In brown seaweeds, the main storage polysaccharides are neutrally charged chains of glucans, built up predominantly by 3-linked β-D-glucopyranose residues known as laminaran (Percival and McDowell 1967; Painter 1983; Kloareg and Quatrano 1988; Lobban and Harrison 1994). On the other hand, structural polysaccharides are more complex; besides the insoluble cellulose, brown seaweeds contain the ionic polysaccharides alginates and fucoidans (Percival and McDowell 1967; Painter 1983; Kloareg and Quatrano 1988). The fucoidans are a very heterogeneous polysaccharides composed of fucose-bearing sulfate groups; they may contain a variable amount of fucose, xylose, galactose, uronic acids, and half ester sulfates (Percival 1968; Medcalf et al. 1978; McCandless and Craigie 1979; Painter 1983; Kloareg et al. 1986; Berteau and Mulloy 2003). Fucoidan in brown algae represents up to 20% of the dry weight (Usov et al. 2001). In algae, its main function is the building of cell walls; it has been suggested also to be enrolled in osmoregulatory activities and protection against desiccation (Black 1954; Percival 1979; Kloareg and Quatrano 1988). Some studies also suggest that fucoidans play a role in the morphogenesis of zygotes of fucoid algae (Hogsett and Quatrano 1978; Bisgrove and Kropf 2001).

Environmental factors which modify the physiology of the algae, also affect the yield and composition of sulfated polysaccharides (SP) (Kloareg and Quatrano 1988; Honya et al. 1999; Usov et al. 2005; Mak et al. 2013; Bruhn et al. 2017). In

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agreement with its function, the fucoidan content is expected to be higher in seaweeds with long periods of exposure to the air, particularly species belonging to the Fucaceae family that inhabit the upper intertidal zone; the closer the algae are to the surface, the greater is their fucoidan content (Black 1954; Kloareg 1981); in the same way, the content and composition of fucoidan differ with the season of harvesting (Skriptsova 2016; Fletcher et al. 2017) and from the part of the thallus where it is obtained (Kloareg and Quatrano 1988; Usov et al. 2005; Skriptsova et al. 2012).

The fucoidan possesses numerous biological properties with potential human health applications (Berteau and Mulloy 2003). Its bioactivity (e.g., antioxidant, anticoagulant, and anticancer) is related to its heterogeneity (Berteau and Mulloy 2003; Ghosh et al. 2009; Ushakova et al. 2009; Fitton et al. 2015; Usoltseva et al. 2018), particularly to the molecular weight; content of fucose, glucuronic acid, and sulfates; and the position of the sulfate groups on the sugar residues (Colliec et al. 1994; Berteau and Mulloy 2003; Li et al. 2005; Zhao et al. 2008; Ale et al. 2011; Kraan 2012).

Silvetia compressa (J. Agardh) E. Serrão, T.O. Cho, S.M. Boo & Brawley (previously known as *Pelvetia fastigiata* (J. Agardh) De Toni) belongs to the Fucaceae family. This is a dominant species in the upper intertidal zone of the temperate waters of the west coast of Baja California (Abbott and Hollenberg 1976; Ricketts et al. 1985; Murray and Bray 1993). The first report of *S. compressa* in the Pacific coast of Baja California was given by Setchell and Gardner (1925) as *Pelvetia fastigiata f. gracilis*. Since then, many floristic studies reported its presence in the temperate Pacific region of the Baja California peninsula (see Pedroche et al. 2008). Up to date, there are no studies reported on SP content of this specie; however, it is expected that *S. compressa* contain SP similar in composition to those species belonging to Fucaceae. *Silvetia compressa* is not currently used in Mexico since there are no chemical and biological studies that justify its commercial harvesting. The aim of the present study is to evaluate the yield and chemical compositions of the SP produced by *S. compressa* and, by fractionation, evaluate the chemical heterogeneity of its fucoidan molecule. Additional analyses were done to evaluate differences among basal and apical tissues and fruiting bodies.

Materials and methods

Silvetia compressa thalli were collected on the intertidal zone of the temperate waters of the Pacific Ocean, along 160 km of Baja California's coast (Fig. 1) where its presence is more conspicuous (Abbott and Hollenberg 1976; Pedroche et al. 2008). Three locations were selected within this region of similar oceanographic conditions based on the access and presence of fishing communities. About 15 whole adult plants were collected by sampling (a total biomass of 3 kg (w/w) when exposed to low tide on three different sites (total distance 160 km). The collected material was dried in a forced air oven at 40 °C, then prior to analysis milled to particle size ≤ 0.5 mm.

Parts of the thallus

From the collected material, subsamples of three complete plants from El Rosario were used to evaluate differences among parts of the thallus: (1) basal part, comprised from the basal disc to the second dichotomies; (2) apical segments, new terminal growth of the plant, without vesicles; and (3) fruiting bodies, considered to be all the vesicles on the terminal dichotomies of the plant. The separated samples were dried in a forced air oven at 40 °C and milled prior to being analyzed.

Sulfated polysaccharide extraction

Samples of 3 g of dry and milled seaweed were extracted in triplicate with 45 mL of 0.2 M HCl in a water bath at 60 °C for 120 min. The solution was centrifuged at 1500 rpm for 5 min, and the remaining particles were re-extracted with 30 mL of 0.2 M HCl at 60 °C for an additional 120 min. The solutions of both extractions were pooled and vacuum-filtered through diatomaceous earth. In order to recover the polysaccharide from the solution, crystals of NaCl were added to 0.1 M and then precipitated with three volumes of concentrated ethanol; the mixture was centrifuged at 1500 rpm and decanted. To remove salts, the precipitated material was washed twice with 70% ethanol, then twice with concentrated ethanol; pressed; and dried at 60 °C, until constant weight.

Fractionation

The extracted sulfated polysaccharides were fractionated by two methods: (1) ethanol-$MgCl_2$ and (2) anion-exchange chromatography.

a) Ethanol fractionation was performed using a modification of the method proposed by Larsen (1978). Briefly, the extracted polysaccharides were precipitated in two ways: (a) 50% ethanol and (b) 50% ethanol with $MgCl_2$ salts (0.1 M), in order to separate soluble and insoluble fractions.
b) Anion-exchange chromatography

Fractionation was performed in a column of 15 × 120 mm, filled with 7.5 g (15 mL) of DE-52 anion-exchange resin. Previous to injection of the sample, the resin was activated by washing with four volumes of 50 mM acetate buffer (pH 5).

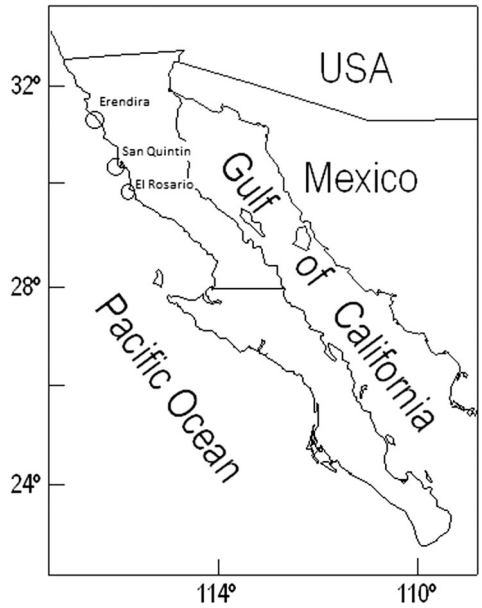

Fig. 1 Sampling sites along the Pacific coast of Baja California, Mexico

A sample of 100 mg of the dry crude extract of *S. compressa* was dissolved in 5 mL of acetate buffer. To elute, first 30 mL of 50 mM acetate buffer was allowed through the column. Then, NaCl solutions in an ascendant step gradient of 0.25, 0.5, 1.0, 1.5, and 2.0 M, 30 mL each, were allowed through the column. With a flow rate of 1 mL min^{-1}, 5-mL fractions were successively collected and characterized.

Chemical analysis

Total carbohydrates in the crude extract were determined colorimetrically by the phenol-sulfuric acid method (Dubois et al. 1956), using D-galactose and L-fucose as standards. Fucose was measured by the method of cysteine for deoxy sugars (Dische 1955), using fucose as standard. Uronic acids were quantified by the modified method of carbazole (Bitter and Muir 1962) using glucuronic acid as standard; and sulfates by the turbidimetric method of barium chloride-gelatin, using K_2SO_4 as standard (Tabatabai 1974; modified by Craigie and Wen 1984).

The contents (%) of fucose, sulfates, and uronic acids were converted to the molar fraction and correlated to fucose as the unit.

FTIR spectra

About 3 mg of the dry extract of each sample was homogenized in solid KBr to form pellets. Their infrared spectra were acquired in the transmission mode in the range of 400 to 4,000 at 4-cm^{-1} resolution and analyzed after 10 scans by Fourier transform using an FTIR-100 Perking Elmer spectrophotometer.

Results

The average SP yield of *S. compressa* from the three sites was 11.4 ± 0.7% in relation to the dry weight of the plant. Yield varied from 11.0 ± 1.0% at the northern locality (Eréndira) to 12.2 ± 0.4% at the southern locality (El Rosario). The sulfate content in the dry extract had a similar pattern with an average of 15.3 ± 0.6% sulfate content (14.8 ± 0.2% in the northern locality to 16.0 ± 0.2% in the southern locality; Fig. 2).

Thallus part

There were differences in SP yield and composition related to the thallus source. The basal part had the higher SP yield (20.7%) and higher sulfate, fucose, and uronic acid contents (5.9, 16.7, and 4.1%, respectively); fruiting bodies had the lower SP yield (9.8%), while the apical part had the lowest sulfate content (2.1%) (Table 1).

Fractionation

The ethanol fractionation with and without $MgCl_2$ yielded two fractions, soluble and insoluble; in both cases, the uronic acids were present only in the insoluble fractions. The soluble fraction was composed only of sulfated fucose. The use of magnesium salts in the fractionation decreases the amount of insoluble fraction with respect to using only ethanol (Table 2).

Four fractions were obtained by ionic exchange chromatography (Fig. 3). A first fraction was eluted with acetate buffer (uncharged molecules). The second fraction appears while increasing the eluent NaCl molarity (0.4–0.8 M). The third and main fraction (at 0.8–1.5 M NaCl) corresponds mainly to fucose with some moiety of uronic acids; and the last fraction (1.5–2.0 M) was composed only of fucose devoid of uronic acids.

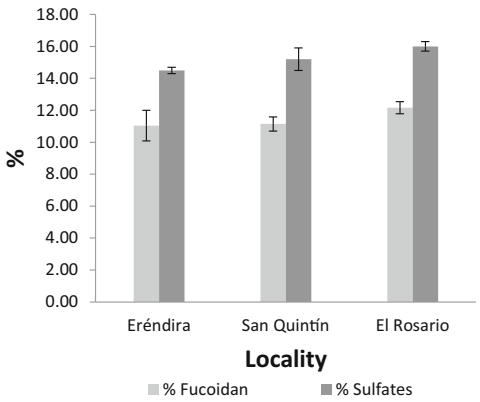

Fig. 2 Fucoidan and sulfate contents in samples of *S. compressa* collected in three different localities of Baja California, Mexico ((1) Eréndira, (2) San Quintín, (3) El Rosario); the vertical lines show the standard deviation ($n = 3$)

Table 1 Average yield and composition of SP from different thallus parts of *S. compressa* (total carbohydrates, fucose, sulfate groups, and uronic acids)

	% of plant dry weight			
Thallus part	CHOS	Fucose	Sulfates	Uronic acids
Fruiting bodies	9.8 ± 0.2	10.0 ± 0.5	4.2 ± 0.8	2.1 ± 0.4
Apical	10.1 ± 0.5	8.7 ± 0.5	2.1 ± 0.2	2.3 ± 0.2
Basal	20.7 ± 0.9	16.7 ± 0.9	5.9 ± 0.1	4.1 ± 0.1

The infrared analysis (FTIR) showed the characteristic signals of sulfated polysaccharides: a strong signal at 1250 cm^{-1} associated to stretching vibration of S=O bond; a signal at 840–850 cm^{-1} associated to axial sulfate group (C-4); additionally, a doublet signal at 1614 and 1417 cm^{-1} generated by the carboxyl groups of uronic acids (Fig. 4a, b). From the fractionation with ethanol in the soluble fractions, there was a small signal at 1417 cm^{-1}, but is absent in the soluble fraction of ethanol/$MgCl_2$ (Fig. 4a); this agrees with the obtained chemical results (Table 2).

Molar ratio

The molar ratio (fucose:sulfates:uronic acids) calculated for the SP of *S. compressa* varied from the less sulfated apical part (1.00:0.65:0.38), to the more sulfated fruiting bodies (1.00:1.20:0.32), while the whole plant was about 1.00:0.98:0.45 (Table 3).

In the same way, the ethanol fractionation modified the molar ratio, where the soluble fraction in ethanol 50% was higher in sulfates but devoid of uronic acids (1:1.12:0.0), while the highest in uronic acids corresponded to the insoluble fraction in ethanol/$MgCl_2$ (1:0.85:1.44) (Table 4).

Discussion

Fucoidan was originally described as sulfated polysaccharides from brown seaweed species such as *Laminaria* and *Fucus vesiculosus* (Kylin 1913). Later studies reported that all the brown seaweeds had sulfated polysaccharides related to fucoidan (McCandless and Craigie 1979; Painter 1983; Berteau and Mulloy 2003). However, the synthesis of fucoidan is species dependent (Honya et al. 1999; Usov et al. 2001; Skriptsova et al. 2012; Ustyuzhanina et al. 2014; Skriptsova 2015; Wang and Chen 2016). Before this study, there are no previous results on the polysaccharides of *S. compressa*.

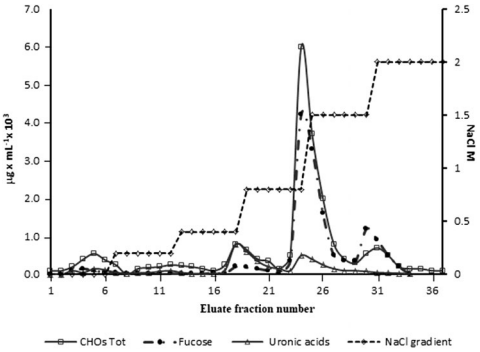

Fig. 3 Fractionation of soluble polysaccharides of *S. compressa* by in DE52 anion-exchange resin in a step gradient molarity of NaCl (0 to 2 M); total carbohydrates, fucose, and uronic acids

The yield of acid SP of *S. compressa* obtained in this study fluctuated between 11 and 12.1% (dw), which is a value within the range of yields reported for related species. Black (1954) found up to 13% fucoidan content in a similar Fucaceae species, i.e., *Pelvetia canaliculata*, and lower fucoidan content (7%) in species growing at a lower water level, *Fucus serratus*. Usov et al. (2001) found that in brown algae, the composition of polysaccharides depends on the species, where seaweeds with a high content of alginates have less fucoidan, especially in Laminariales. However, in a Fucaceae species, i.e., *Fucus evanescens*, they reported considerable amounts of alginates and fucoidan (17.3 and 7.7%, respectively). Larsen (1978) reported a fucoidan content of 6–8% in dw in *Ascophyllum nodosum*, 9–11% in *Fucus* species, and 5–20% in *Laminaria* sp., but in *P. canaliculata*, they found a fucoidan content of 20% in dw.

Several studies have reported that the content and composition of SP in brown seaweeds differ with the part of the thallus (Kloareg and Quatrano 1988; Usov et al. 2005; Skriptsova et al. 2012; Skriptsova 2016). In this study, the older tissue (basal) showed the highest fucose yield and sulfate yield (16.7 and 5.9%, respectively), indicating the highest fucoidan content, while the apical part had the lowest (8.7%). Our results are concordant with those reported by

Table 2 Fractionation of sulfated polysaccharides of *S. compressa* in 50% ethanol and 50% ethanol/0.1 M $MgCl_2$

Fraction	Media	% of total	Fucose	Sulfates	Uronic acids
Soluble	Ethanol	44.7	16.9 ± 0.1	10.4 ± 0.03	0.00
	Ethanol/$MgCl_2$	54.8	44.5 ± 0.6	22.2 ± 0.8	0.00
Insoluble	Ethanol	55.3	19.7 ± 0.2	8.5 ± 0.04	33.0 ± 0.2
	Ethanol/$MgCl_2$	45.2	18.9 ± 0.6	8.8 ± 0.3	33.4 ± 0.4

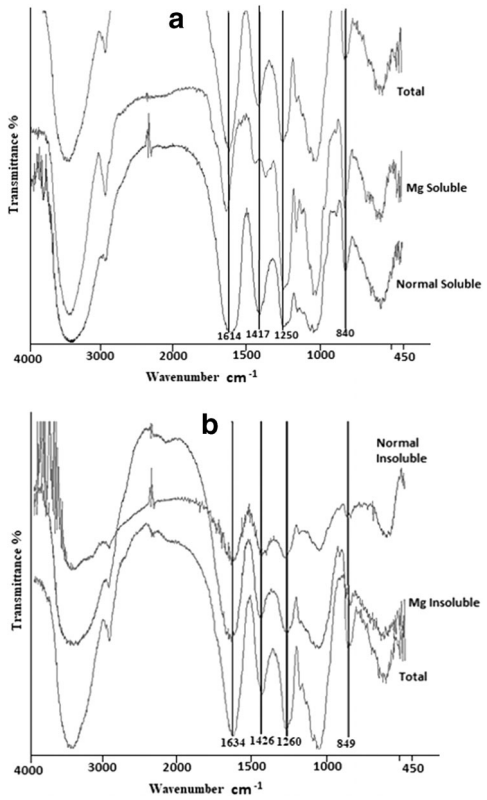

Fig. 4 FTIR spectra for fractions of fucoidan from *S. compressa* obtained by precipitation in ethanol at 50% and ethanol at 50% with $MgCl_2$: **a** soluble and **b** insoluble fractions

Zvyagintseva et al. (2003) who found a higher fucoidan content in older plants of *Laminaria cichorioides* compared with young ones. In contrast, other studies in brown seaweeds have shown that the reproductive tissue has higher fucoidan content (Usov et al. 2005; Skriptsova et al. 2012; Mak et al. 2013). Skriptsova et al. (2012) reported that fertile plants of *Silvetia babingtonii* have relatively high fucoidan content (25% dry weight). Some studies showed that in the non-reproductive thallus, these fronds contain more fucoidan than stipes and midribs (Black 1954; Usov et al. 2005). Our results suggest that the proportion variation of SP of the different parts of the plant varies with the species.

As previously referred, the extraction method influences the yield and composition of the extracted polysaccharides (Berteau and Mulloy 2003; Ale et al. 2011). Thus, in the original extraction method (Kylin 1913), the acid extract could be composed of a mixture of sulfated polysaccharides, some fragment of uronic acids, and free sugars (Ale and Meyer 2013; Bruhn et al. 2017). The employed acid strength (0.2 N) was intended to avoid the concomitant extraction of alginates and proved to extract more sulfated polysaccharides than did water alone or $CaCl_2$ media (data not shown).

The heterogeneity of the extracted SP of *S. compressa* was demonstrated by precipitation with ethanol and ethanol/$MgCl_2$ (Larsen 1978) and by anion-exchange chromatography. The ethanol precipitation rendered soluble and insoluble fractions, where the soluble fraction was bigger with the use of magnesium salts than when using only ethanol, while the insoluble fraction was lower. In both conditions, i.e., ethanol and ethanol/$MgCL_2$, the soluble fraction was devoid of uronic acids, while the insoluble was enriched in uronic acids (Table 2). Thus, the obtained composition of the soluble fraction could be compared with the strict fucoidan definition (Larsen et al. 1966; Larsen 1967, 1978; Mian and Percival 1973; Hogsett and Quatrano 1975; Bilan et al. 2002).

On the other hand, the anion-exchange chromatography applied to the SP of *S. compressa* showed that the extracted polysaccharides in acid media corresponded to a mixture of different charged molecules, where four fractions were obtained (Fig. 3). It is noticeable that the fucose-containing polysaccharides (fractions 3 and 4) represent more than 90% of the extracted polysaccharides and must be the more sulfated molecules (data non-available). Similar results were obtained for commercial fucoidan by Patankar et al. (1993), who separated fucoidan fractions differing in sulfate content. Other studies found a similar pattern in crude fucoidan extract of *F. evanescens* (Bilan et al. 2002) and in the hydrolyzed extract of *Hizikia fusiforme* (Wang et al. 2012)

In agreement with our results, FTIR analysis showed the characteristic signals of sulfated polysaccharides: a strong signal at 1240–1250 cm^{-1} for total sulfates (S=O stretching vibration) (Pereira et al. 2013); a signal at 840–850 cm^{-1} due to axial sulfate group in C-4 position; this is in accordance with the fact that the main sulfate group for fucoidan of different brown seaweeds is positioned in C-4 as occurs in *Chorda filum* (Chizhov et al. 1999), *Sargassum stenophyllum*

Table 3 Molar ratio of components in sulfated polysaccharides from different thallus parts of *S. compressa*

Thallus part	Fucose	Sulfates	Uronic acids
Fruiting bodies	1.0	1.20	0.32
Apical	1.0	0.65	0.38
Basal	1.0	0.95	0.36
Whole	1.0	0.98	0.45

Table 4 Molar ratio of the components (fucose:sulfates:uronic acids) obtained of the fractions of fucoidan from *S. compressa*, separated by precipitation in ethanol and ethanol with 0.1 M $MgCl_2$

Fraction	Media	Fucose	Sulfates	Uronic acids
Soluble	Ethanol 50%	1.00	1.12	0.00
	Ethanol 50%/$MgCl_2$ 0.1 M	1.00	0.90	0.00
Insoluble	Ethanol 50%	1.00	0.78	1.37
	Ethanol 50%/$MgCl_2$ 0.1 M	1.00	0.85	1.44

(Duarte et al. 2001), and *Laminaria saccharina* (Cumashi et al. 2007). Other signal is a doublet at 1620 cm^{-1} and 1416 cm^{-1} indicating the asymmetric and symmetric stretching vibrations of carboxylate (RCOO-) (Synytsya et al. 2003; Silva et al. 2005; Wang and Chen 2016). On the other hand, the absence of uronic acids in the soluble fraction is detected by changes in the signal at 1417 cm^{-1} only in the FTIR spectra of ethanol with $MgCl_2$ (Table 2, Fig. 4a).

Finally, the molar ratio of components of the fucoidan of *S. compressa* showed that this polysaccharide is composed mainly of fucose with high proportion of sulfates (from 0.6 to 1.1 mol by mol of fucose) and the uronic acids (from 0.3 to 1.44 mol per mol of fucose) (Tables 3 and 4). Bilan et al. (2002) found a molar ratio of fucose:sulfates of 1:1.23 for fucoidan of *F. evanescens*. The fractionation with ethanol yields molecules with 1 mol of sulfate for each mole of fucose (Table 4) in agreement with the fucoidan from *Nemacystus decipiens* (Tako et al. 1999).

Conclusions

The polysaccharides isolated in acidic conditions from the brown seaweed *S. compressa* are fucoidan type, composed of a mixture of fucose with high sulfate content and fractions of uronic acids. While the yield of SP was relatively constant along the sampled region, the content and composition varied between the tissue sources, where a higher content was obtained for basal tissue but higher sulfation occurs in fruiting bodies. The crude SP extracted from *S. compressa* can be separated in molecules containing sulfated fucose with uronic acids and molecules with only sulfated fucose.

The obtained molar ratio showed that in the sulfated polysaccharide of *S. compressa*, each mole of fucose corresponds almost 1 mol of hemiester sulfate and, based on FTIR spectra, the fucoidan of *S. compressa* is mainly sulfated in C-4 (axial sulfate group). Thus, this sulfation pattern is more related to Laminariales species than to Fucales. This study provides the bases for potential use of *S. compressa* as a source of fucoidan. It is important to point out that before any attempts to consider this species for commercial purposes, population and biological studies would be necessary in order to define sustainable harvesting potential.

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