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Extraction of sulfated polysaccharides by autohydrolysis of brown seaweed *Fucus vesiculosus*

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Abstract The extraction of sulfated polysaccharides (fucoidan) by autohydrolysis (AH) of brown seaweed *Fucus vesiculosus* was studied. Experimental assays were performed under different conditions of temperature (160 to 200°C) and reaction time (10 to 30 min) according to a 2^2 central composite design, and the conditions able to maximize the fucoidan yield were selected. The alga degradation and the total sugar yield in the liquor after AH were also determined to each experimental condition. The highest fucoidan yield (~16.5% *w*/*w*) was obtained when the AH process was performed at 180°C for 20 min. This product was characterized by high-performance liquid chromatography, infrared analysis spectroscopy, and thermal gravimetric analyses, which verified the presence of fucose and galactose as main components (70:30% mol ratio, in average) and an SO_3 content higher than 20%. AH process under optimum reaction conditions was an effective method to recover fucoidan from *F. vesiculosus*. The use of this technology brings also important advantages from economical and environmental viewpoints since it does not require the use of chemical solvent and generates less waste when compared to conventional extraction procedures.

Keywords Algae · *Fucus vesiculosus* · Autohydrolysis · Sulfated polysaccharides · Optimization

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Introduction

In recent years, great attention has been given to the use of marine seaweed biomass. Such interest has been supported by important advantages that the use of this kind of biomass represents: (a) low future fluctuations in biomass demand are expected due to overpopulation; (b) feasibility of growing fast in the open ocean; (c) higher photosynthetic efficiency than terrestrial biomass; (d) no limitation by water and to a lesser extent by temperature; and (e) low costs of collection (Ross et al. 2008; Anastasakis et al. 2011). Main components of marine seaweeds differ from that of terrestrial biomass (cellulose, hemicellulose, and lignin) and include phytochemically active molecules such as polysaccharides, fatty acids, proteins, vitamins, and mineral elements, which are compounds with potential applications in food, cosmetic, pharmaceutical, and medical fields (O'Sullivan et al. 2010; Anastasakis et al. 2011; Lordan et al. 2011).

Brown seaweed-derived polysaccharides such as fucoidan, laminaran, alginates, and mannitol have been studied due to their effectiveness as anticoagulant, antitumor, antithrombotic, anti-inflammatory, contraceptive, and antiviral agent (Bhakuni and Rawat 2005; Imbs et al. 2009; Wang et al. 2009; Mestechkina and Shcherbukhin 2010). Fucoidans, or sulfated fucans, may constitute up to 25–30% of the seaweed dry weight, depending on the seaweed species and,

to a lesser extent, on life history stage and season (Kusaykin et al. 2008). The most relevant biological functionalities of these compounds are the activities against hepatitis, herpes, and human immunodeficiency (AIDS) viruses, anticoagulant heparin inflammation, cell proliferation and adhesion, and fertilization functions (Ellouali et al. 1993; Berteau and Mulloy 2003; Queiroz et al. 2008).

The extraction of sulfated polysaccharides from seaweeds has been usually performed by using hot water, dilute acid, or dilute alkali—all of these methodologies involve long extraction time and high volume of diluents (Duarte et al. 2001; Marais and Joseleau 2001; Rioux et al. 2007; Yang et al. 2008; Skriptsova et al. 2010). Among the existing technologies of hydrolysis, the autohydrolysis (AH) is an eco-friendly process that could be an interesting alternative for application on the recovery of biological compounds from seaweeds. This process requires only the use of water as extraction solvent, and the hydronium-catalyzed reactions of the material fibers proceed through water autoionization at elevated temperatures (150–230°C). This process offers several important advantages, such as: (1) simple and economical operation; (2) elimination of corrosive problems; (3) mild operational conditions for selective degradation of the biomass; and (4) generation of low concentrations of sugar degradation products in the media (Garrote et al. 1999).

Despite of all the above-mentioned benefits, research on the application of AH process to macroalgae is limited. The aim of the current study was to evaluate the extraction of sulfated polysaccharides (fucoidan) by AH of brown seaweed *Fucus vesiculosus*. An experimental design was proposed to evaluate the effect of the process variables (temperature and reaction time) on the responses: fucoidan yield, alga degradation, and total sugar yield in the liquor after extraction. The product obtained was characterized to determine the monosaccharide and sulfate contents. Infrared analysis spectroscopy and thermal gravimetric analyses were also performed to explain the characteristics of the extracted fucoidan.

Materials and methods

3,5-Dinitrosalicylic acid was purchased from Fluka, Chemika; anthrone reagent was from Prolabo, Normapur, Merck; and Coomassie Plus (Bradford) Assay Kit was obtained from Thermo Scientific Co. Other reagents were all of analytical grade.

Samples of *F. vesiculosus* were collected from Praia Norte (Viana do Castelo, Portugal) during the spring season (May 2010). The seaweed was washed with fresh water in order to remove salt, sand, and epiphytes, dried at 35°C, milled in a home blender, and stored in plastic bags at room temperature. Particles lower than 1,000 μm were not used in the experiments. The seaweed sample had moisture and ash contents of 14.84±0.83% (*w*/*w*) and 17.5±0.25% (*w*/*w*), respectively (dry weight basis).

Autohydrolysis process

For the extraction of sulfated polysaccharides from *F. vesiculosus*, 2 g of milled seaweed was suspended in 50 mL of distilled water (alga/water ratio of 1:25), and the mixture was placed in 160-mL stainless steel cylinder reactors, which were submerged in an oil bath with open heating circulator (Julabo Labortechnik GmbH, Germany) and PID temperature control. The autohydrolysis process was carried out under different conditions of temperature and reaction time (Table 1). At the end of the reaction, the reactors were removed from the oil bath and immediately immersed in an ice bath to stop the reaction. The obtained suspension was vacuum-filtrated to separate the liquor from the residual alga, which was dried at 35°C, weighed to determine the percentage of alga degradation ($\%AD_{AH}$), and stored. Total sugar yield in the liquor after AH ($\%TS_{LAH}$) was quantified, and subsequently, a 1% (*w*/*v*) solution of $CaCl_2$ was added to the liquor in a ratio of 1:1 (*v*/*v*) for alginate removal (4°C overnight storage). Free alginate liquor was recovered by filtration in qualitative paper, and then double volume of ethanol absolute was added to the resultant filtrate, and the mixture was stored at 4°C for 8 h. The precipitated polysaccharide ($\%Fuc_{AH}$) was recovered by centrifugation (8,500 rpm, 15 min, 4°C), dried at 35°C, milled, and stored for analyses. Two replicates of each experiment were carried out.

The fucoidan yield ($\%Fuc_{AH}$), alga degradation ($\%AD_{AH}$), and total sugar yield in the liquor after extraction ($\%TS_{LAH}$) were calculated by using Eqs. (1), (2), and (3), where WM_{OH} is the dry mass weight obtained after ethanol precipitation; WA is the alga weight used in each experiment; WA_{AH} is the dry alga weight recovered after AH; TS_{AH} is the mg of total sugar in the hydrolysates obtained after AH; and TS_A is the mg of total sugars in the alga *F. vesiculosus* (35.12 mg TS/100 mg alga).

$$\%Fuc_{AH} = \frac{WM_{OH}}{WA} \times 100 \quad (1)$$

$$\%AD_{AH} = \left(\frac{WA - WA_{AH}}{WA}\right) \times 100 \quad (2)$$

$$\%TS_{LAH} = \left(\frac{TS_{AH}}{TS_A}\right) \times 100 \quad (3)$$

Table 1 Values of the process variables, severity factor ($\log R_0$), and results of fucoidan yield ($\%Fuc_{AH}$), alga degradation ($\%AD_{AH}$), and total sugar yield in the liquor after extraction ($\%TS_{LAH}$), to each experimental condition used for autohydrolysis of *Fucus vesiculosus*

Process variables—real and (coded) values					Responses		
Run	Temperature (°C)	Reaction time (min)	$\log R_0$[a]	Final pH	$\%Fuc_{AH}$	$\%AD_{AH}$	$\%TS_{LAH}$
1	200 (+1)	30 (+1)	4.4	4.90	4.58±1.31	30.52±1.45	14.49±0.72
2	200 (+1)	10 (−1)	4.0	6.57	8.22±1.07	61.62±5.39	8.62±1.20
3	160 (−1)	10 (−1)	2.8	6.79	3.23±0.08	73.82±2.60	3.66±0.17
4	160 (−1)	30 (+1)	3.2	6.31	6.05±1.14	61.42±0.46	11.06±2.16
5	180 (0)	10 (−1)	3.4	6.73	5.68±0.32	66.32±4.46	6.47±0.72
6	180 (0)	30 (+1)	3.8	5.13	8.42±1.49	34.09±0.83	14.92±0.12
7	200 (+1)	20 (0)	4.3	5.01	7.16±0.87	32.67±1.84	11.38±1.67
8	160 (−1)	20 (0)	3.1	6.52	7.16±0.86	61.55±3.54	7.16±0.31
9	180 (0)	20 (0)	3.7	6.15	18.14	47.35	13.35
10	180 (0)	20 (0)	3.7	6.20	16.57	45.77	14.89
11	180 (0)	20 (0)	3.7	6.09	15.34	43.67	13.98
12	180 (0)	20 (0)	3.7	6.41	15.88	44.25	15.89

[a]The severity parameter of the autohydrolysis process was calculated by Eq. (4)

The severity parameter ($\log R_0$) of the AH process was calculated by Eq. (4) (Overend and Chornet 1987), where t is the time (min) at the temperature of reaction T (°C), and 14.75 is an empirical parameter related with the temperature and activation energy.

$$R_0 = \int_0^t \exp\left[\frac{T(t) - 100}{14.75}\right] dt \quad (4)$$

In a subsequent step, sequential extraction assays were performed aiming to verify the possibility of reusing the residual alga (after the AH process) to maximize the fucoidan recovery yield. Sequential extraction procedures were performed under the previously established AH conditions (180°C, 20 min), following the same procedure described above. After the first extraction (E1), the residual solid material was separated by filtration, resuspended in distilled water to reach the alga/water ratio of 1:25 (*w*/*v*), and hydrolyzed again (E2) in the oil bath system.

Analytical procedures

For characterization of the recovered fucoidan, samples of 10–15 mg of the fucoidan extracts were hydrolyzed with 4 N HCl (2 mL) at 121°C for 2 h. The total sugar content was then measured by the anthrone method using glucose as standard (Ludwig and Goldberg 1954), and the sulfate group content was determined by turbidity with barium chloride–gelatin method (Dodgson 1961). All absorbance measurements were carried out in triplicate. Protein was determined by the method of Bradford (1976) and the total phenolic compounds by the method of Folin–Ciocalteau.

The concentration of monosaccharides was determined by hydrolysis of the fucoidan samples (10–15 mg) with 2 M trifluoroacetic acid (0.5 mL) at 121°C for 2 h, in glass tubes sealed with N_2. Hydrolyzed polysaccharides were cooled in an ice water bath, centrifuged at 5,000 rpm for 5 min, and the liquid fraction was neutralized to pH 7 with 2 M NaOH. The resulting samples were injected in a high-performance liquid chromatography (Jasco, Japan) system equipped with a low-pressure gradient solvent pump, an autosampler with 20-μl loop, and a refraction index detector (Jasco, Tokyo, Japan). Samples were injected in a MetaCarb 67H (300× 7.8 mm) column at 60°C, using 0.005 M H_2SO_4 as mobile phase at a flow rate of 0.5 mL min^{-1}. Micrographs of the seaweed samples were obtained in a scanning electron microscope Nova NanoSEM 200 (Netherlands) using the samples sputtered with gold under high-vacuum conditions and an accelerating voltage of approximately 15 kV.

Thermal gravimetric analysis (TGA) was performed in a thermo gravimetric analyzer model TGA-50 (Shimadzu Corporation, Japan) under nitrogen atmosphere. Differential scanning calorimetry (DSC) analysis was performed using a Modulate DSC-50 (Shimadzu Corporation, Japan). Mass samples of 10–13 mg were run from room temperature to 600°C at a rate of 10°C min^{-1}.

Infrared analysis spectroscopy (FTIR) was carried out on a Perkin-Elmer 16 PC spectrometer, using 16 scans and frequency range of 400–4,000 cm^{-1}. For the analysis, the polysaccharide was ground with potassium bromide powder and then pressed into 1-mm pellets. The vibration transition frequencies of each spectrum were baseline corrected and the absorbance was normalized between 0 and 1.

Experimental design and data analysis

The effects of the independent variables, temperature (X_1, °C) and residence time (X_2, min), on the extraction of fucoidan by AH were evaluated through a 2^2 central composite design with four replicates at the center point.

The variables were coded according to Eq. (5), where x_i is the coded value of the variable X_i, X_0 is the value of X_i at the center point, and ΔX_i is the step change. The real and coded values of the variables are shown in Table 1. Experimental runs were randomized to minimize the effects of unexpected variability in the responses.

$$x_i = (X_i - X_0)/\Delta X_i \quad (5)$$

The fucoidan yield (%Fuc_{AH}), alga degradation (%AD_{AH}), and total sugar yield in the liquor after extraction (%TS_{LAH}) were taken as responses of the experimental design. The results were analyzed by analysis of variance, and the behavior of the system was explained by Eq. (6), where Y is the dependent variable, β_0 is constant, β_i, β_{ii}, and β_{ij} are the coefficients estimated by the model, and X_i and X_j are the levels of the independent variables. Analyses of the experimental data were carried out using the software STATISTICA™ v 6.0 (Statsoft®, USA).

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad (6)$$

Results and discussion

Sulfated polysaccharides yields

The fucoidan yield (%Fuc_{AH}), alga degradation (%AD_{AH}), and total sugar yield in the liquor after extraction (%TS_{LAH}) to each experimental AH condition are shown in Table 1. As can be seen, great variations occurred in all these responses according to the used process condition. The fucoidan yield, for example, was increased when the value of the severity factor ($\log R_0$) was increased, but up to a certain limit [$\log R_0$=3.7 (180°C and 20 min; runs 9–12)−%Fuc_{AH}= 15–18% *w*/*w*]; after which, a significant lost of yield was observed [$\log R_0$=4.4 (200°C and 30 min; run 1)−%Fuc_{AH}= 4.6% *w*/*w*]. The pH decrease observed in the reaction media obtained by using the highest values of severity factor may have affected the fucoidan recovery, since acid media could have favored the hydrolysis of this polysaccharide, causing a decrease in the final recovery yield.

The highest values of total sugar yield in the liquor (>14% *w*/*w*) were obtained when using $\log R_0$ similar or higher than 3.7. This result suggests that the sugars obtained at 180°C for 20 min (runs 9–12) probably are polysaccharides of long chain able to agglomerate and precipitate as sulfated fucans, causing an elevated fucoidan recovery yield. On the contrary, it is also possible that the total sugars obtained at 200°C for 30 min (run 1) are mainly monosaccharide and/or short chain polysaccharides, explaining the low recovery yield.

The effect of the AH process over brown seaweed fucoidan may be attributed to the heterogeneous backbone structure of this molecule, with alternating (1→3) and (1→4)-linked 2- and/or 4-sulfated-α-L-Fucρ residues. The depolymerization of this structure affects the (1→3)-α-L-Fucρ residues faster than the (1→4)-α-L-Fucρ residues, causing intermolecular changes with transference of the sulfate groups to pentose and hexose sugars, which allow obtaining polysaccharides and oligosaccharides of multisulfated (up to three) fucans (Anastyuk et al. 2010). Therefore, all the fucoidan samples recovered in the current study presented high sulfate content (>18%).

The highest fucoidan yield (~16.5% *w*/*w*) obtained in the present study was similar to the values reported by microwave-assisted extraction of *F. vesiculosus* (Rodriguez-Jasso et al. 2011) and was higher than the values reported by diluted HCl treatment of *Laminaria cichorioides* (Anastyuk et al. 2010) or by sequential extraction with $CaCl_2$ and HCl from either *F. vesiculosus*, *Ascophyllum nodosum*, or *Saccharina longicruris* (Rioux et al. 2007). In the last cases, besides the higher yield, AH has also other important advantages when

Table 2 Effect estimates (EE), standard errors (SE), and level of significance (p) for the responses of fucoidan yield (%Fuc_{AH}), alga degradation (%AD_{AH}), and total sugar yield in the liquor after extraction (%TS_{LAH}), obtained by autohydrolysis of *Fucus vesiculosus*

Variables	%Fuc_{AH}		%AD_{AH}		%TS_{LAH}	
	EE±SE	p	EE±SE	p	EE±SE	p
x_1	1.17±2.34	0.634	−23.99±2.94	0.000[b]	4.20±1.31	0.019[a]
x_1^2	−10.85±3.51	0.021[a]	8.49±4.42	0.103	−6.50±1.97	0.017[a]
x_2	0.64±2.34	0.793	−25.24±2.94	0.000[b]	7.24±1.31	0.001[b]
x_2^2	−11.07±3.51	0.020[a]	14.68±4.42	0.016[a]	−3.65±1.97	0.114
x_1x_2	−3.23±2.87	0.303	−9.35±3.60	0.041[a]	−0.77±1.61	0.651

x_1 temperature, x_2 reaction time

[a] 95%, significance level

[b] 99%, significance level

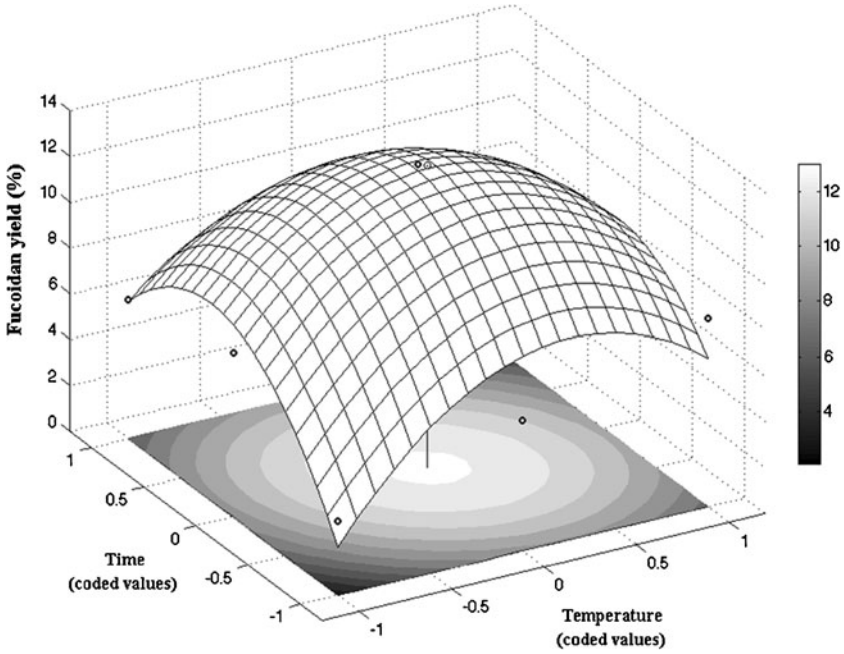

Fig. 1 Response surface fitted to the experimental data points corresponding to the fucoidan yield during autohydrolysis of *F. vesiculosus* under different conditions of temperature and reaction time

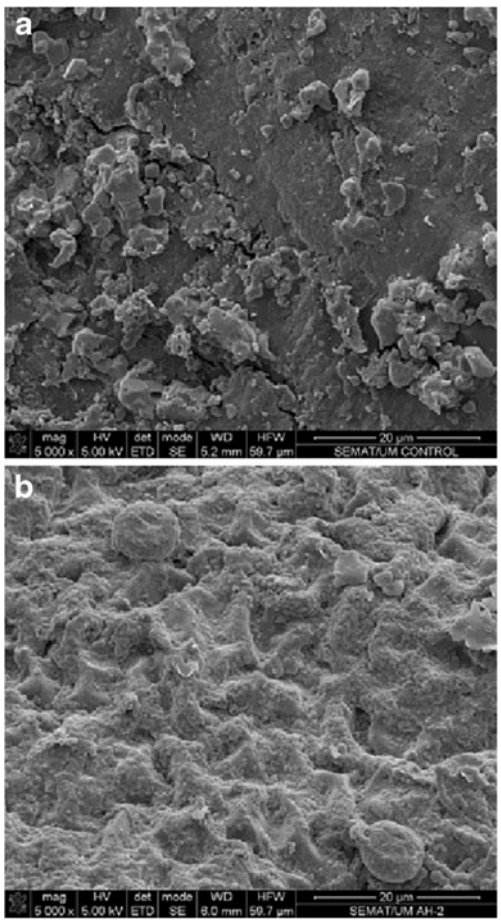

Fig. 2 Scanning electron micrographs of *F. vesiculosus*: **a** untreated sample; **b** residual sample obtained after autohydrolysis at 180°C for 20 min. Magnification ×5,000-fold

compared to these extraction methods. Since it does not require the use of chemicals as an extraction solvent, it is more environmentally friendly, easier to operate, and does not cause corrosion problems in the equipment (Garrote et al. 1999). On the other hand, when compared to microwave-assisted extraction, which also used only water as extraction solvent and promoted similar fucoidan extraction yield in a short time of process (1 min), an economical analysis would be useful to verify if AH would be more economically viable to extract fucoidan from brown seaweed. In any way, AH could be more easily used on an industrial scale, while the scale-up of microwave-assisted extraction systems is an important barrier to be overcome for its industrial application (Mandal et al. 2007).

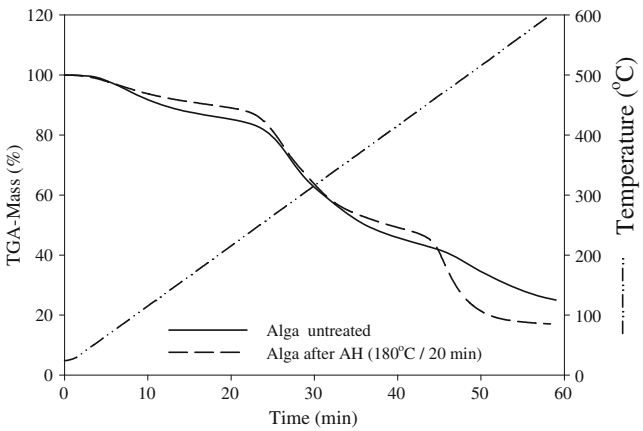

Fig. 3 TGA of *F. vesiculosus* before and after the autohydrolysis process under optimized reaction conditions (180°C, 20 min)

Table 3 Chemical composition of fucoidan samples recovered by autohydrolysis of *Fucus vesiculosus* under different operational conditions

Run	Autohydrolysis condition		Fucoidan composition					
	Temperature (°C)	Time (min)	Fucose (% mol)	Galactose (% mol)	SO_3 (%)	TS/SO_3[a] (mol mol^{-1})	Protein (mg L^{-1})	Phenols[b] (%)
1	200	30	52.21	47.79	30.78± 2.13	1/1.82	5.55±1.03	3.15±0.53
2	200	10	83.96	16.04	21.02± 2.33	1/0.96	5.11±0.83	3.69±0.16
3	160	10	81.16	18.84	23.67± 1.40	1/1.33	4.97±0.17	4.84±0.16
4	160	30	75.08	24.92	25.59± 2.42	1/1.14	5.51±0.31	5.38±0.06
5	180	10	74.12	25.88	22.08± 1.16	1/0.95	10.39±2.13	3.95±0.21
6	180	30	55.14	44.86	20.96± 2.51	1/0.79	10.90±1.00	4.87±0.46
7	200	20	51.79	48.21	19.06± 0.44	1/1.00	12.41±0.36	3.99±0.91
8	160	20	77.75	22.25	18.46± 0.66	1/1.08	9.21±1.37	3.35±0.01
9–12[c]	180	20	76.76	23.24	21.21± 0.76	1/0.87	8.56±0.57	5.63±0.78

Monosaccharide amounts are expressed as the percent of total sugar content in the sample

TS total sugars

[a] TS/SO_3=(mg TS/100 mg fucoidan)/(mg SO_3/100 mg fucoidan)

[b] mg of total phenols/100 mg of fucoidan

[c] Mean value of four center point assays

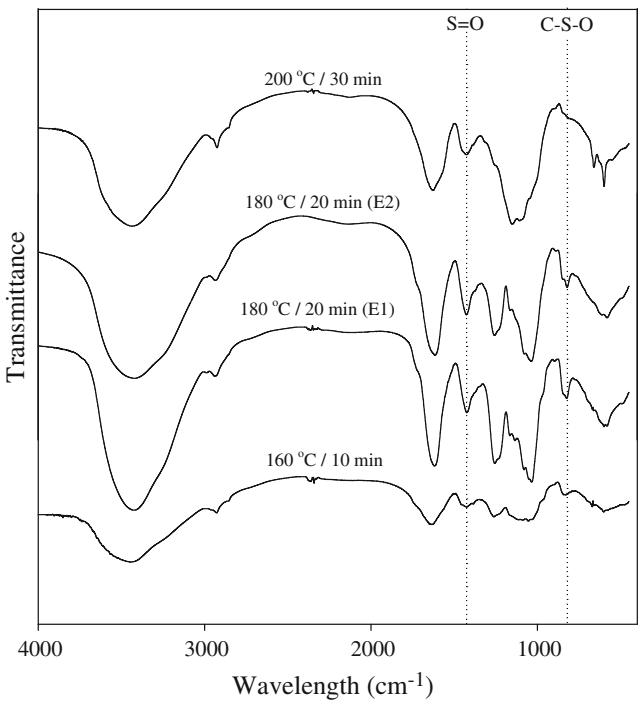

Fig. 4 FTIR of fucoidan samples obtained by autohydrolysis of *F. vesiculosus* under different operational conditions

Statistical analysis and optimization of autohydrolysis conditions

The effect estimates of the operational variables as well as their significance level on the responses are shown in Table 2. As can be noted, the studied variables significantly affected all the responses, presenting individual (first and second order) and/or interaction effects. A three-dimensional response surface was plotted in order to

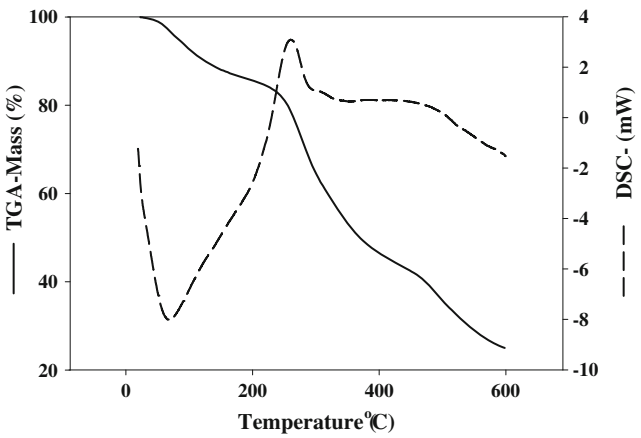

Fig. 5 TGA and DSC of the fucoidan sample recovered under the optimized autohydrolysis conditions (180°C, 20 min)

represent the fucoidan yield variations as a function of the temperature and reaction time variations. This figure (Fig. 1) clearly shows that the fucoidan yield was not linearly increased when the process variables were increased, but there was an optimum point after which the use of higher temperature and reaction time did not improve the yield. This is in agreement with the analysis presented in Table 2, which revealed significant effect of the quadratic term of both variables on the fucoidan yield response. An estimate of the critical point revealed that 180°C and 20 min were the conditions able to maximize the fucoidan yield.

The alga degradation and the total sugar yield in the liquor after extraction were also affected by the quadratic terms of the variables (Table 2). This fact suggests that similar to the observed for the fucoidan yield, the variation of these responses did not occur linearly by increasing the value of the variables. A regression analysis was then performed to fit equations able to predict the value of the responses according to the temperature and reaction time variations used in the present study. The mathematical models expressed in Eqs. (7), (8), and (9) (coded values of the variables) represent the fucoidan yield (Y_1), alga degradation (Y_2), and total sugar yield in the liquor after extraction (Y_3), as a function of the temperature (x_1) and reaction time (x_2) used during the AH. Such models were established with high coefficient of determination R^2 being able to explain between 84 and 97% of the responses variations.

$$Y_1 = 15.18 + 0.59x_1 - 5.43x_1^2 + 0.32x_2 - 5.54x_2^2 - 1.62x_1x_2 (R^2 = 0.84) \quad (7)$$

$$Y_2 = 44.46 - 12.00x_1 + 4.25x_1^2 - 12.62x_2 + 7.34x_2^2 - 4.68x_1x_2 \quad (R^2 = 0.97) \quad (8)$$

$$Y_3 = 13.86 + 2.10x_1 - 3.25x_1^2 + 3.62x_2^2 - 1.82x_2^2 - 0.38x_1x_2 \quad (R^2 = 0.91) \quad (9)$$

Figure 2 shows the alga structure before and after the AH process under the optimized conditions (180°C for 20 min). As can be seen, the original (untreated) sample exhibited a rigid and ordered surface (Fig. 2a), which was modified after the AH process (Fig. 2b), becoming more porous and rough due to the removal of components from this structure. The TGA profiles of these samples (untreated and AH-treated alga) (Fig. 3) presented similar behavior, with three stages in the degradation pathway. The first stage (<215°C) basically corresponds to dehydration of the sample; the second one (215–490°C) consists in the devolatilization of the sample, and the third stage (>470°C) corresponds to the decomposition region, with the remaining mass at the end of this stage being correspondent to the ash (mineral) content in the sample. High mineral content (~26% *w*/*w*) was present in the untreated alga, and a considerable amount of this fraction (~17% *w*/*w*) remained in the alga after the AH process. These results allow concluding that the AH process was more selective for the extraction of fucoidan than minerals from *F. vesiculosus*.

After optimizing the AH conditions, a sequential extraction process was evaluated as a strategy to maximize the fucoidan yield. By using this sequential process of AH, low fucoidan yield (4.47% *w*/*w*) was obtained in the second extraction step. This strategy was then considered unviable, since the involved energy costs would not justify the little increase in the extraction yield.

Characterization of the extracted fucoidans

Chemical composition

Chemical composition of fucoidans significantly varies according to species, growth conditions, and extraction procedure, and for this reason, elucidating the chemical composition of polysaccharide's samples is very important in order to understand the influence of extraction processing on extractive products. The fucoidan samples obtained in the current study presented a heterogeneous structure mainly composed of fucose and minor proportions of galactose (Table 3). In most of the cases, the concentration of fucose was higher than 75% mol. Presence of other monosaccharides including xylose and glucose was not verified in any fucoidan sample.

High sulfate content (>20%) was found in practically all the recovered fucoidans (Table 3). This is an advantageous aspect since sulfate groups have been reported to present important biological functions such as anti-HIV activity; and such activity is potentially increased when the sulfate content is increased (Schaeffer and Krylov 2000). Additionally, all the samples presented a ratio between total sugars and sulfate content (TS/SO_3) higher than 1/0.5 mg TS/mg SO_3 and small quantities of proteins and phenolic compounds (Table 3). As a whole, chemical composition of the recovered fucoidan samples is comparable to other reports of sulfated fucans obtained from different sources using different extraction procedures such as sequential extraction of *F. vesiculosus* and *A. nodosum* by $CaCl_2$ and HCl (Rioux et al. 2007), hydrothermal (autoclaving) hydrolysis of *Laminaria japonica* (Wang et al. 2009), extraction of *L. cichorioides* by HCl (Anastyuk et al. 2010), and microwave-assisted extraction of *F. vesiculosus* (Rodriguez-Jasso et al. 2011). In these cases, fucoidan was also mainly constituted by fucose (with minor amounts of galactose and/or manose) and presented sulfate contents varying between 20 and 30% *w*/*w*.

FTIR analysis

FTIR spectra of the fucoidan recovered under the optimized AH conditions, as well as other sulfated fucans samples extracted by AH (Fig. 4), showed typical absorption bands of fucoidans. IR bands at 1,200–970 cm^{-1} are mainly caused by the C–C and C–O stretching vibrations in the pyranoid ring and C–O–C stretching of the glycosidic bonds. Intense absorption in this region is common for polysaccharides. However, the absorption band at 1,240–1,255 cm^{-1} (S=O stretching) confirms the presence of sulfate in the recovered polysaccharides. The band at 840 cm^{-1} suggests a complex pattern of substitution of α-linked L-fucopyranose at the axial C-4 position, whereas those at 833–820 cm^{-1} are associated to low amounts of substitution at the equatorial C-2 and C-3 position (Marais and Joseleau 2001; Wang et al. 2010).

Thermal analysis

TGA and DSC curves of fucoidan extracted under the optimized AH conditions are shown in Fig. 5. Similar profile for sulfated fucans is reported by Rodriguez-Jasso et al. (2011); however, in the present study, the volatile matter evolution for AH-fucoidan presented a constant decrease of mass without showing the stationary stage in the decomposition region. This fact suggests that the use of higher temperature (>600°C) would be required to reach this stage. In any way, it is possible to verify that the fucoidan sample contained low quantity of mineral compounds (sulfates, phosphates, and carbonates).

In brief, autohydrolysis process was a suitable technology for extraction of sulfated polysaccharides from *F. vesiculosus*. The use of this technology brings important advantages from economical and environmental viewpoints since when compared to conventional extraction procedures, the autohydrolysis method is more environmentally friendly (it does not require the use of chemical solvent and generates less waste). The current study revealed that the fucoidan yield as well as the fucose and sulfate contents in the polysaccharide were affected by the temperature and reaction time used for autohydrolysis. Optimization of the extraction conditions was a useful strategy to maximize the fucoidan yield (~16.5% *w*/*w*).

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